Vol. XLII No. 10

October, 1953

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

1852 APhA 1953
SECOND CENTURY

CONTENTS OF THIS ISSUE

Pharmacological Properties of a New Antihistaminic Agent, Phenyltoloxamine (Bristamin) 587
Justin B. Hoekstra, Dorothea E. Tisch, Nathan Rakieten, and H. L. Dickison

Antitubercular Substances. III. Nonpyridinoid Heterocyclic Hydrazides 594
Robert I. Meltzer, Arnold D. Lewis, Freeman H. McMillan, Jerome D. Genzer, Frederick Leonard, and John A. King

The Effect of Antipyretics on the Erythrocytic Sedimentation Rate of Rats 600
Duane G. Wenzel, Jack L. Beal, and Albert R. Haskell

Oleanolic Acid from Cranberries 602
Betty Y. T. Wu and Lloyd M. Parks

Chemical Studies on Ursolic Acid 603
Betty Y. T. Wu and Lloyd M. Parks

Observations on the Antispasmodic Principle of *Euphorbia pilulifera*, L. 607
Floyd P. Hallett and Lloyd M. Parks

Trihexyphenidyl—Experimental Studies Employing a Muscle-Nerve Preparation 609
O. D. Priddle, Jr. and Y. T. Oester

The Antitubercular Activity of D-Galacturonic Acid Isonicotinyl Hydrazone 612
Peter P. T. Sah and S. Anderson Peoples

Tolerance to Bacterial Pyrogens in the Rabbit 614
David M. Tennent and Walther H. Ott

Effect of Panparnit on the Oxygen Uptake of Rat Brain 618
Laslo Kajdi and Clara H. Williams

The Spectrophotometric Determination of Phenylacetone in Amphetamine 621
Stanley K. Freeman

Tolerance, Toxicity, and Caloric Availability of Intravenously Injected Oxypolygelatin Solution in Rabbits 623
Lawrence E. Detrick, Virginia Debley, and Thomas J. Haley

Separation of Some of the Opium Alkaloids by Surface Chromatography 627
Mitchell L. Borke and Ernst R. Kirch

The Use of Chelating Agents for Accelerating Excretion of Radioelements 629
Harry Foreman

Symposium on Antibiotics 632

Anesthesia. XLIII. The Estimation of Anesthetic Trifluoroethyl Vinyl Ether in Aqueous Solution 633
Frederick K. Bell and John C. Krantz, Jr.

Digitoxin 635
Paul Demoen and Paul Janssen

1954 Iodine Research Award Nominations Requested 641

Book Notices 642

Advertising ..... Cover pages 2, 3, 4, and pp. I–IV

Copyright 1953, by the American Pharmaceutical Association

Literature citations should be grouped at the end of the manuscript under the heading "References." All bibliographic references should be checked with the original publications. The citations should be numbered consecutively with full-sized Arabic numerals enclosed in parentheses corresponding to the numbers used in the text. The names of all periodicals cited in the list of references must be abbreviated in accordance with abbreviations given by **Chemical Abstracts** in its "List of Periodicals Abstracted." The sequence followed in the citations must be as follows:

(1) Doe, J. B., *Am. J. Physiol.*, **79**, 289(1927).

References to books should be in the order given, (1) author's surname and initials, (2) title of the book, (3) volume, (4) edition, (5) name of publisher, (6) place of publication, (7) year of publication, and (8) page number. A complete book reference may be illustrated as follows:

(1) Gilman, H., "Organic Chemistry," Vol. 2, 2nd ed., John Wiley and Sons, New York, 1943, pp. 1236, 1252.

Arabic numerals must be used to designate all definite weights, measurements, percentages, and degrees of temperature unless occurring at the beginning of a sentence, in which case the numeral and measurement are spelled out. Periods of time should be written in words. Unusual characters and Greek letters should be written plainly and legibly or should be explained by notes on the margin of the page. Fractions should be written with a slant line. Standard abbreviations should be used whenever weights and measures are stated in the metric system, e. g., 10 Gm., 2.5 cc., etc. The forms to be used are: cc., Kg., Gm., mg., mm., cm., and L.

Figures.—All drawings should be made in India ink, preferably on white tracing paper or cloth. Original drawings are preferable, but clear, well-prepared glossy photographic prints are acceptable. The size of illustrations should not exceed the 8.5″ x 11″ manuscript size. They should be designed for reduction to the width of one column (2.75″) or full page width (5.5″). Thus, height rather than width should be accentuated where possible.

Numbers and measurements, etc., which are to appear on the axes of the drawing should be indicated in pencil outside the axes, in order that they may be set in type. Captions and legends should be placed *below* the diagram (not on it), or, if lengthy, collected on a separate typewritten list, identifying each by its proper figure number and including whatever acknowledgments are necessary.

Photographs should never be rolled, and paper clips should not be used unless guarded by a piece of paper folded several times to prevent an indentation in the photograph. All drawings and photographs should be protected from bending and from the chance of having their edges torn while in transit. Do not write on the back of a photograph. Any indentation in the photograph, however slight, will be likely to show as a blemish in the reproduction. All figures submitted must be referred to in the text of the manuscript, and should be numbered consecutively with Arabic numerals, e. g., Fig. 1, Fig. 2, etc. Kymograph tracings should not be submitted with the original experimental legends which are likely to have been hastily or carelessly inscribed. Authors may find it worth while to employ a professional draftsman to make the drawings in proper form for reproduction.

Tables.—Tables should be carefully constructed so that the data presented may be understood easily. The indiscriminate use of tables should be avoided. Tables should be constructed so as to occupy fully a single column (2.75″) which will take about 40–50 characters and spaces. Tables which require two columns are also acceptable. Interpretative statements should not be written on the body of a table but should be explained in footnotes. Column heads should be made as small as possible and standard abbreviations should be used liberally. Data which may easily be deduced by simple arithmetic from data in another column should not be included. All tables should be referred to in the text of the manuscript. Every table and every column should be supplied with an appropriate heading. It is not necessary to place tables on separate sheets, and in fact it is preferable to intersperse them in the manuscript. Tables should be numbered consecutively using Roman numerals. The table number and the title should be placed in a continuous heading above the data presented. The preparation of a clear and concise table of data requires much planning and infinite care.

STAFF PHARMACIST

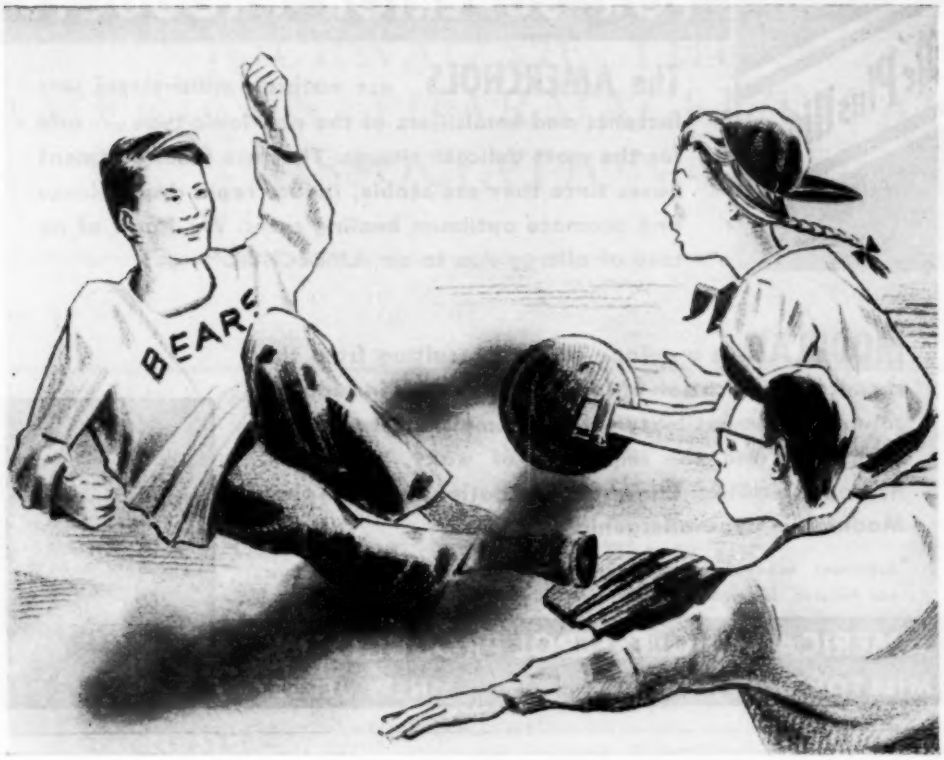
Eligible for registration in Texas. Experience not necessary. Woman preferred.

Write to: C. R. Coleman, University of Texas, Medical Branch, Galveston, Texas

Guarantee their future NOW!

Pharmacy offers greater opportunities now, as never before, for interesting and successful careers for young men and women who complete B.Sc. degree courses at this institution, oldest yet most modern of its kind in the Americas. M.Sc. and D.Sc. degrees, and courses in Schools of Bacteriology, Biology and Chemistry also. Founded in 1821. Write for free catalog.

Philadelphia
COLLEGE OF PHARMACY AND SCIENCE
43rd St., Woodland and Kingsessing Aves., Philadelphia 4, Pennsylvania



SCORE ON BASES MADE WITH VEEGUM

JUST OFF THE PRESS, Bulletin No. B122 contains the latest technical information on VEEGUM.

VEEGUM is an anhydrous powdered ointment base, easily hydrated in cold water. Viscosity ranges from thin lotions to stiff ointments by simple adjustment of the water content. VEEGUM dispersions are compatible with a wide variety of active ingredients.

VEEGUM combines with other bases to form opaque, washable preparations. It increases the viscosity and suspending ability of polyethylene glycol ointments. VEEGUM blends with petrolatum to give non-tacky, smooth-spreading ointments. Its combined emulsifying and suspending ability make VEEGUM particularly valuable in formulations containing sticky ingredients or those difficult to disperse.

VEEGUM is inorganic, non-toxic, and non-irritating. It is highly purified Colloidal Magnesium Aluminum Silicate.

WRITE TODAY for the complete VEEGUM story.

R. T. VANDERBILT CO.
SPECIALTIES DEPARTMENT
230 Park Avenue New York 17, N.Y.

☐ Please send VEEGUM bulletin No. B122.
☐ Please send sample of VEEGUM.

NAME_____

POSITION_____

(Please attach to, or write on, your company letterhead)

FOR SUPERIOR PRODUCTS

The AMERCHOLS® are natural, multi-sterol surfactants and emulsifiers of the non-ionic type . . . safe for the most delicate tissues. They are ideal ointment bases since they are stable, induce rapid drug release and promote optimum healing rates. We know of no case of allergy due to an **AMERCHOL.***

MODULAN[T. M.] is a unique product resulting from the chemical modification of lanolin. Properties include: oil solubility; compatibility with o/w emulsions, soaps and shampoos; and the imparting of waxy, protective, hydrophobic films. Clinical investigations indicate that Modulan is hypo-allergenic.*

**References, technical data and suggested formulations are available from our research laboratories. Please write on your business letterhead.*

AMERICAN CHOLESTEROL PRODUCTS, INC.
MILLTOWN • • • NEW JERSEY

Scale illustrated is our
No. 540 all Glass Model

Capacity—4 oz. (120 grams)
Sensitivity—1/10 grain
Finish—Genuine light mahogany with chrome plated parts
Price—$135.00 (subject to change without notice)

PROMPT DELIVERY
on this as well as other class A scales

No. W35 (all glass in lid) $100.00
No. W33 . . . . . 90.00
All With Stainless Steel Pans

Our scales have no bands or wires to stretch or break

Still the best and lowest priced scale made

HENRY TROEMNER
Manufacturer of the Finest Scales for the Druggist Since 1840
911 Arch Street
Philadelphia 7, Pa.
(Send for Circular A-A)

The Massachusetts College of Pharmacy

Longwood Avenue, Boston

Graduate study in Boston has always appealed strongly to students in the fields of medicine and public health. The environment is stimulating and attracts students from all parts of the world. Library, laboratory, hospital, and other facilities for graduate work are outstanding in this area.

The Massachusetts College of Pharmacy is located in this great medical and educational center. It offers unusual opportunities to graduates of colleges of pharmacy who are interested in further study in pharmacy, pharmaceutical chemistry, and pharmacognosy.

For additional information, write to HOWARD C. NEWTON, Dean

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLII — *OCTOBER, 1953* — NUMBER 10 — CONSECUTIVE NO. 20

Pharmacological Properties of a New Antihistaminic Agent, Phenyltoloxamine (Bristamin)*,†

By JUSTIN B. HOEKSTRA, DOROTHEA E. TISCH, NATHAN RAKIETEN,‡ and H. L. DICKISON§

Bristamin, N,N-dimethyl-2-(α-phenyl-*o*-toloxy)-ethylamine (generic name, phenyltoloxamine) has been found to be a potent antagonist to many of the pharmacologic responses of histamine. Direct comparisons of this material with tripelennamine and diphenhydramine gave very favorable results. In addition to *in vitro* studies specific antihistaminic properties were demonstrated in guinea pigs and dogs. Local anesthetic properties were shown in rabbits and guinea pigs. LD_{50} results (in mg./Kg.) following intraperitoneal injections into mice were: phenyltoloxamine hydrochloride 163, phenyltoloxamine dihydrogen citrate 246, diphenhydramine 114, and tripelennamine 79. Chronic toxicity studies were done in rats and dogs. Dogs tolerated doses (calculated as base) of 20 and 40 mg./Kg. of phenyltoloxamine dihydrogen citrate for one year with no untoward effects and no evidence of blood dyscrasia. In addition to its antihistaminic properties, phenyltoloxamine possesses some antispasmodic properties and distinct local anesthetic properties. It is concluded that phenyltoloxamine in the form of its salts is a very effective antihistaminic agent and is one of the least toxic of such agents reported to date.

THE REPORTS by Ungar, Parrot, and Bovet (1), Bovet and Staub (2), and Staub (3) that certain phenolic ethers were effective histamine antagonists started a search for other compounds which would be equally or more effective since it was soon found that the most active compound in this series, thymoxyethyldiethylamine (929F), had serious toxic effects.

In a preliminary screening of the alkylaminoalkyl ethers of the benzyl phenols prepared by Cheney, Smith, and Binkley (4), Goodman and Loewe (5) found that one of the compounds, 2-benzylphenyl β-dimethylaminoethyl ether or N,N-Dimethyl-2-(α-phenyl-*o*-toloxy)-ethylamine was a more active histamine antagonist than diphenhydramine.[1] We have given this compound the name Bristamin and the generic name phenyltoloxamine. Independently, Mills, Rohrmann, Dinwiddie, and Lee (6) reported similar findings. Recently, Hisamoto (7) compared this compound with eight other antihistaminic agents and stated that its overall pharmacological properties were better than the others. This compound has been studied by other investigators under the Bristol number C5581H and Lilly numbers 01500 and 01780.

Since this compound possesses a high antihistaminic potency and is among the least toxic of such compounds, it seems desirable at this

* Received April 2, 1953, from the Department of Pharmacologic Research, Bristol Laboratories Inc., Syracuse, New York.

† Bristamin is Bristol Laboratories Inc. trade-mark name for N,N-Dimethyl-2-(α-phenyl-o-toloxy)-ethylamine.

‡ Present Address: South Shore Analytical Laboratories, South Islip, Long Island, New York.

§ The authors wish to express thanks to Miss Dorothea S. Fisher for valuable technical assistance.

[1] Throughout this paper the generic names diphenhydramine and tripelennamine are used instead of the trademark names Benadryl and Pyribenzamine.

time to present a fairly complete study of its pharmacologic properties. Both the dihydrogen citrate and hydrochloride salts have been used in this study. Where the name phenyltoloxamine is used in the experimental section below it should be understood that the hydrochloride salt was actually used unless otherwise stated and that the doses used are expressed as weight of the salt. The hydrochloride salt has a tendency to be hygroscopic, hence, the dihydrogen citrate has been used in commerce. The clinical use of this compound will be reported by Hoople and Brewer (8) and Seyler and Simon (9).

EXPERIMENTAL

Specific Antihistaminic Properties

In Vitro.—The ability of the compound being studied to prevent a histamine-induced spasm or to break a barium chloride induced spasm was studied by a modification of the method of Magnus (10). Strips of guinea-pig ileum were used in the study involving histamine induced spasms and strips of rabbit ileum were used in the study involving barium chloride induced spasms. Papaverine was the reference standard. The strips were suspended in a modified Ringer-Tyrodes solution at a temperature of 37.5°. Under these conditions a 5×10^{-6} concentration of histamine diphosphate produces a spasm which can be prevented by a 5×10^{-5} concentration of papaverine hydrochloride. Similarly, a 2.5×10^{-5} concentration of papaverine hydrochloride will abolish a spasm produced by a 5×10^{-4} concentration of barium chloride. All concentrations in this section are expressed as milligrams per milliliter. Using phenyltoloxamine hydrochloride instead of papaverine hydrochloride and assigning the papaverine concentration a value of 1, a preventative index of 100 and a lytic index of 1.0–2.0 was obtained. Under these same conditions, diphenhydramine had a preventive index of 20 and tripelennamine one of 10.

In the same type of an experiment atropine sulfate in a concentration of 1.25×10^{-5} broke the spasm induced in a strip of rabbit ileum by a concentration of 5×10^{-7} of acetylcholine chloride. The concentration of phenyltoloxamine which will abolish such an acetylcholine spasm was one hundred times greater than that for atropine sulfate.

The same types of experiments as described above were also done employing uterine strips from virgin guinea pigs. In these experiments the antihistaminic agent being described had an atropine index (lytic) of <0.00025 and a papaverine index of <1.0 with respect to preventive activity against histamine.

In Vivo.—*Histamine Aerosol.*—Guinea pigs were exposed to an atmosphere containing atomized histamine diphosphate fifteen minutes after an intraperitoneal injection of the test compound (11). Thirty guinea pigs in addition to the control animals were used in this study with phenyltoloxamine. The controls uniformly died. Tripelennamine and diphenhydramine were both studied in the same fashion. The doses protecting 50% of the test animals from death due to histamine asthma were found to be 0.06 mg./Kg. for tripelennamine, 0.19 mg./Kg. for phenyltoloxamine, and 0.22 mg./Kg. for diphenhydramine.

Blood Pressure Studies.—These studies were carried out on dogs under pentobarbital anesthesia. Blood pressure was recorded from the carotid artery and injections of drugs were made into the femoral vein. In some instances the antihistamine was given by stomach tube. After determining a given dog's normal responses to the following drugs: histamine diphosphate (0.003 and 0.02 mg./Kg.), acetylcholine chloride (0.003 and 0.5 mg./Kg.), epinephrine hydrochloride (0.001 mg./Kg.), arecoline hydrobromide (0.05 mg./Kg.) and pilocarpine hydrochloride (0.1 mg./Kg.), a dose of 0.1 mg./Kg. of the antihistaminic agent was given. The responses to the above test drugs were again determined. Using the same dog, the dose of antihistamine was increased stepwise from the above dose; after each dose the responses to the test drugs were determined. A suitable interval of time was allowed between administrations of all drugs to allow the blood pressure to assume a steady state.

The blood pressure response following injections of the control agents was expressed as a per cent of the normal response when no antihistaminic agent had been given. For example, if a given dose of histamine produced a fall in blood pressure equivalent to 60 mm. of mercury before the antihistamine was given and a fall equivalent to 30 mm. after the antihistamine was given, then the per cent of normal response is 50. The results of intravenous administration are shown in Table I. Figure 1 shows a record of the above type of experiment.

These data show that as little as 0.5 mg./Kg. of phenyltoloxamine intravenously was effective in partially blocking the vasodepressor response to histamine; a dose of 10 mg./Kg. by the same route blocked to about 60% on an average. Some blocking action was exhibited against acetylcholine and arecoline especially at high doses. It required 5.0

TABLE I.—EFFECT OF INTRAVENOUS PHENYLTOLOXAMINE ON ALTERATIONS IN BLOOD PRESSURE FOLLOWING INJECTION OF HISTAMINE, ACETYLCHOLINE, EPINEPHRINE, AND ARECOLINE

	Effect on Blood Pressure Expressed as Per Cent of Normal Response					
	Histamine		Acetylcholine		Epinephrine	Arecoline
Dose, Mg./Kg.	0.003 Mg./Kg.	0.02 Mg./Kg.	0.003 Mg./Kg.	0.5 Mg./Kg.	0.001 Mg./Kg.	0.05 Mg./Kg.
0.1	88.7[a]	98[b]	76[a]	100[c]	82.7[a]	100[c]
0.5	61[a]	60[c]	59.7[a]	99[c]	87[a]	101[c]
1.0	53.5[d]	54.7[a]	54.7[d]	100[c]	80.7[d]	101[c]
5.0	57.5[d]	47.2[d]	68.7[d]	105.3[a]	82.7[d]	72.5[b]
10.0	39.5[d]	42.7[d]	61[d]	102.2[d]	92.7[d]	87.7[d]

[a] Average of results from three animals.
[b] Average of results from two animals.
[c] One determination.
[d] Average of results from four animals.

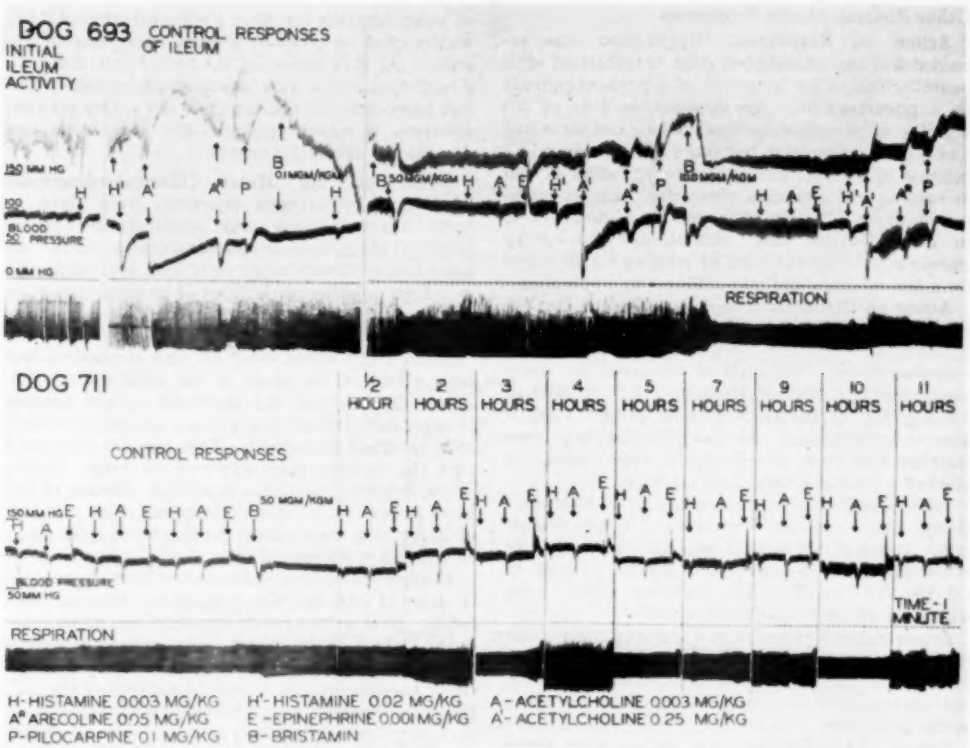


Fig. 1.—Effect of phenyltoloxamine on alterations in blood pressure of pentobarbitalized dogs following injections of histamine, acetylcholine, and epinephrine. The top tracing in addition shows the effect following injections of arecoline and pilocarpine as well as the effect on alterations in the activity of the ileum.

mg./Kg. orally to accomplish some degree of blocking to histamine. Moreover, even with a dose of 20 mg./Kg. orally at the end of one and one-half hours the effect is not as great as with a dose of 5 mg./Kg. intravenously.

A special variation of the above procedure was employed as a screening technique for antihistaminic agents. After obtaining the control responses to intravenous injections of histamine, acetylcholine, and epinephrine, a single intravenous dose of 5 mg./Kg. of the antihistaminic agent was given. Following this the responses to the control drugs were determined up to five hours at half-hour intervals. The per cent deviation of the given response from the average normal response (in absence of antihistaminic agent) was used as the criterion of effect. After collecting such data for several antihistaminic agents, they were then analyzed statistically by a sequential plan of analysis (12).[2] Selecting one of the agents as a standard the other could be compared with it.

The above analysis indicated it was not possible to say that phenyltoloxamine was better than tripelennamine at one hour but it was possible to state with statistical significance (error 5%) that it was not poorer than tripelennamine. The same situation held at three hours. The total number of dogs used in this study was ten.

Duration of action studies carried out on two dogs showed that there was good blocking action up to six hours after an intravenous dose of 5 mg./Kg. From six to ten hours after injection there was a variable and decreasing degree of blocking and at eleven to twelve hours after injection the responses were again normal.

Small intravenous doses of this compound have very little direct effect on blood pressure. Larger doses (10–20 mg./Kg.) have a very pronounced depressor action to the extent of as much as 50% of the existing blood pressure. Doses greater than 20 mg./Kg. will reduce the blood pressure to practically zero.

Action on Intestinal Tract.—Dogs were placed under pentobarbital anesthesia and the lower ileum was attached to an internal organ recording device as described by Jackson (13). These studies were done in the same dogs as were used for the studies on circulatory effects, and the intestinal responses were therefore observed at the same time as those on blood pressure. Phenyltoloxamine tends to decrease the tone and activity of the dog's ileum. This compound has definite blocking action against the responses of the ileum to histamine, acetylcholine, arecoline, and pilocarpine in doses of 1.0 mg./Kg. and greater.

[2] We are deeply indebted to Dr. M. A. Brumbaugh and Mr. R. Hofstead, Statistical Department of Bristol Laboratories Inc., for bringing this method of analysis to our attention and applying it to our results. Details of the application to biological problems will be published elsewhere.

Other Pharmacologic Properties

Action on Respiration.—Respiration was recorded kymographically on dogs anesthetized with pentobarbital either by means of a tracheal cannula or a pneumograph. An intravenous dose of 5.0 mg./Kg. of phenyltoloxamine in some instances had no effect on respiration but more often produced an increase in the rate and force of respiration as well as causing a decrease in chest size. Larger intravenous doses (15.0 to 30.0 mg./Kg.) administered to unanesthetized dogs produced a respiratory embarrassment in the form of gasping which lasted for a few minutes and was followed by hyperpnea.

Action on Uterus in Situ.—*Dog.*—In one dog the uterus was carefully separated from the ovarian ligament and attached by means of a thread to a recording lever. The method employed for study was essentially that of Barbour (14). A dose of 5.0 mg./Kg. of phenyltoloxamine caused a slight drop in uterine tone and also initiated large contractions and more or less regular tone waves. It blocked a pitocin spasm about one-third.

Rat.—Using the same technique as described above, this compound was studied in five female rats. Doses of 1.0 to 10.0 mg./Kg. had very little effect upon the unstimulated uterus. Doses of 1.0 to 10.0 mg./Kg. did, however, cause some relaxation of pitocin induced spasms.

Action on the Eye.—*Systemic Administration.*—In studies with two unanesthetized rabbits, doses of 5.0 and 10.0 mg./Kg. of phenyltoloxamine dihydrogen citrate, injected intravenously, had no effect on the pupil size.

Topical Administration.—In studies with three rabbits, three drops each of a 1.0, 5.0, and 10.0% solution had no effect on the pupil size.

Action on Salivary Glands.—A dose of 10.0 mg./Kg. blocked the secretion of saliva induced by a dose of 0.1 mg./Kg. of pilocarpine in one out of four dogs anesthetized with pentobarbital. Both drugs were given intravenously.

Irritant Action.—One, five, and ten per cent solutions were placed in the conjunctival sac of the rabbit's eye. These solutions had a *p*H of 5.0–6.0. The 5 and 10% solutions produced redness, swollen lids, and tearing. This irritation persisted for some forty-eight hours. Three rabbits were used in this study.

Sedative Effects.—Phenyltoloxamine when tested by the method of Winter (15) has very slight sedative potential. This potential has not been found to any great extent in clinical studies (9).

Local Anesthetic Effects.—Phenyltoloxamine dihydrogen citrate was tested for local anesthetic effects by instilling a few drops of 0.01 to 5.0% solutions into the eyes of rabbits or guinea pigs, then observing whether the corneal wink reflex was abolished following stroking of the cornea. Additional tests were done employing subdermal injection into the shaved backs of guinea pigs and determining whether or not the response to a given electrical stimulus was abolished.

It was found a 0.1% solution produced local anesthesia of the cornea of rabbits and guinea pigs of short duration but that a concentration of 0.5% was needed to produce a local anesthesia of the guinea pig skin following subdermal injection. In direct comparison with procaine, phenyltoloxamine had approximately the same potency. One per cent solutions of either produced the same type and duration of anesthetic effects.

Effect on the Heart (Electrocardiographic Studies).[1]—Intravenous injections were given to both unanesthetized and anesthetized (pentobarbital) dogs and electrocardiograms made. In some instances only single injections were made and in others repetitive injections of either constant or varying amounts were employed. All dogs were intact except one in which the vagi were cut.

Small intravenous doses of this compound had little effect on the heart of the anesthetized dog. Doses up to 5.0 mg./Kg. produced a slight increase in heart rate (ten to twenty beats per minute) which soon returned to normal. This may be associated with the vasodepressor action of the drug. Larger doses, however, produced a definite slowing of the heart in both intact and vagotomized dogs. A dose of 20 mg./Kg. may reduce the heart rate to as much as one-half of its normal rate.

Electrocardiograms showed very little change until doses of 10.0 mg./Kg. and greater were administered. Under these conditions there was an increase in the voltage of the *R* and *T* components as well as the appearance and accentuation in the voltage of the *S* component and a marked sagging of the *ST*. Heart conduction was also slightly slowed. With very large doses there was dissociation in the heart with only a ventricular rhythm. There was rapid disappearance of electrical activity as the heart stopped beating with no fibrillation. Recovery to a fairly normal electrocardiogram was quite rapid after the administration of nonlethal doses.

In the unanesthetized dog large doses produced a marked tachycardia (up to about three hundred beats per minute). The heart also went from a marked sinus arrhythmia to a regular rhythm. Lethal doses produced terminal slowing of the heart, dissociation, and rapid disappearance of electrical activity. Figure 2 shows the records on one dog.

It is noteworthy that anesthesia alters the response; with anesthesia the rate slows, without anesthesia the rate quickens. In each case there was respiratory difficulty. It may be that anesthesia only eliminates the "protective" reflex by which anoxia induces tachycardia.

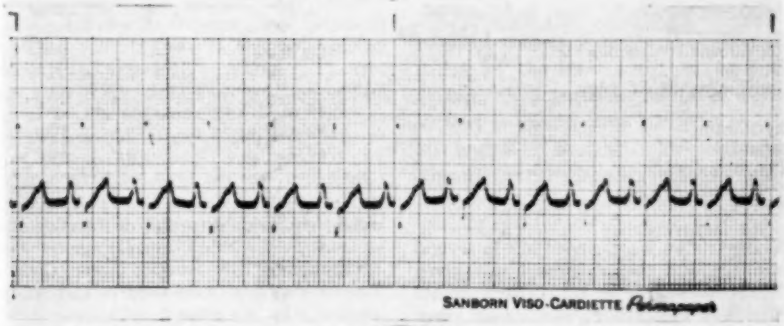
Most of the effects seen after the intravenous injections of this drug are similar to those seen in anoxia. The slowing of the heart is apparently not due to vagal stimulation since the vagotomized dog behaved in the same manner. The rapid disappearance of electrical activity and the absence of fibrillation, however, are not typical of simple anoxia.

---

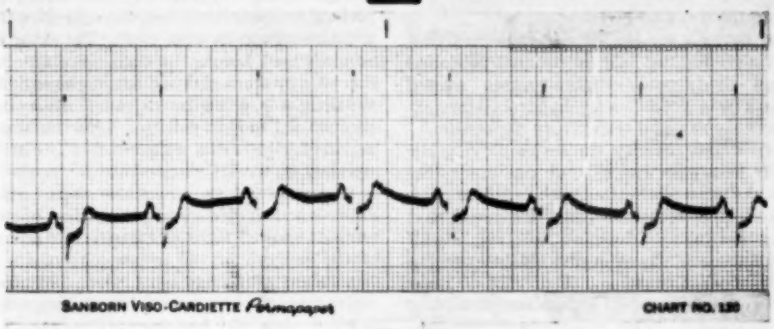
[1] We wish to thank Dr. Jane S. Robb, Pharmacology Department, State University of New York Medical Center at Syracuse, for her kind assistance in analyzing these electrocardiograms.

Fig. 2.—Electrocardiograms following injections of increasing amounts of phenyltoloxamine until death occurred. Lead II was employed throughout. *1.* Control before injection of phenyltoloxamine. *2.* After 50 mg./Kg. *3.* After 70 mg./Kg. *4.* After a lethal dose had been given. →

1


SANBORN VISO-CARDIETTE Pulmograph

2


SANBORN VISO-CARDIETTE Pulmograph
CHART NO. 120

3

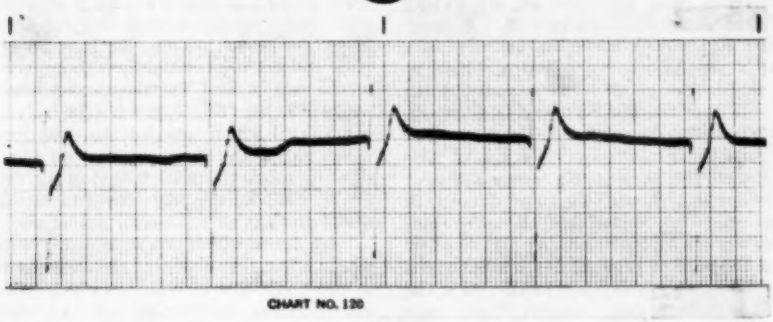


CHART NO. 120

4

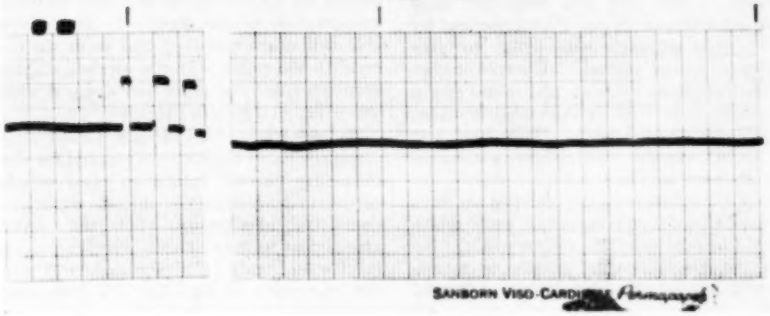

SANBORN VISO-CARDIETTE Pulmograph

TABLE II.—ACUTE TOXICITY—MICE

Drug	LD_{50} Mg./Kg./Mouse Oral	I. P.	I. V.	Slope	No. Mice
Phenyltoloxamine hydrochloride	424 ± 54			8.5	26
		163 ± 10		24	28
			33 ± 2	21	40
Phenyltoloxamine dihydrogen citrate		246 ± 11			35
Diphenhydramine		114 ± 6		15	60
Tripelennamine		79 ± 5		16	60

Toxicity

Acute.—These studies were carried out in several species of animals and the compound was given either as the hydrochloride or dihydrogen citrate salt. The results of such studies in mice are given in Table II. A calculation of the LD_{50} was done by the deBeer graphic method (16). For comparison purposes acute toxicities of tripelennamine and diphenhydramine are also given.

Acute oral toxicity studies in rats indicated that doses up through 680 mg./Kg. of the hydrochloride produced no symptoms. Higher doses caused death. Death was preceded by hyperactivity, excitement, convulsions, and respiratory depression.

Acute intravenous toxicity studies employing the hydrochloride salt were carried out on unanesthetized dogs and dogs anesthetized with pentobarbital. The studies on unanesthetized dogs indicated that the animals could tolerate doses up to 30 mg./Kg. if this dose were given slowly or in divided doses with a short time interval in between. A dose of 20 mg./Kg. injected rapidly produced generalized muscular twitches and spasms. The animal was semiconscious for one-half hour but recovered. A dose of 30 mg./Kg. injected rapidly caused almost immediate death. Death was preceded by respiratory spasms and generalized muscular spasms. In anesthetized dogs continuous intravenous injection of a 1% solution of phenyltoloxamine hydrochloride at the rate of 0.2 cc. every ten seconds caused death when the total dose reached 68.2 mg./Kg. in one dog and 89.2 mg./Kg. in another. The latter dog was vagotomized. Intravenous injection of this material at the rate of 10.0 mg./Kg. every half hour required a total dose of 80 mg./Kg. to produce death in two dogs, one vagotomized and the other not, while in another nonvagotomized dog a dose of 96 mg./Kg. was required to produce death when given at the rate of 20 mg./Kg. every half hour. As in the case of unanesthetized dogs, smaller doses were lethal when the material was injected rapidly.

Subchronic.—Young mature male and female rats weighing between 116 and 194 Gm. were divided into two groups of ten each and were fed daily except Sunday at levels of 10 and 20 mg. per rat by stomach tube. No attempt was made to give equivalent dosages to the animals. Control studies were done simultaneously with an additional group of rats. Animals were kept in metabolism cages and urinary output was followed. Daily food and water intake of the animals were roughly estimated and the animals were weighed daily.

The study was terminated after thirty-two days and a male and female survivor from each group was sacrificed for post-mortem examination. No pathology of the viscera either grossly or microscopically could be found. All animals showed weight gains equal to that of the control. Urinary secretion was within normal range.

Chronic.—These studies were done in dogs. Twenty-one dogs were used in this study which is divided into two phases.

First Phase (Hydrochloride Used for this Study).—Twelve dogs were fed the hydrochloride salt at dosage levels varying from 10–400 mg./Kg. for periods ranging from two days to fifty-three weeks and one served as a control. The drug was always administered before the dogs were fed. Dosing at first was by stomach tube but at the end of the study the drug was given by capsule. Animals were fed commercial food in pellets. This was supplemented at varying intervals with meat. The animals were weighed at weekly intervals.

Post-mortem examinations were made in all animals which died during the course of study. The animals showed signs of gastritis and some irritation of the mucosa of the upper part of the intestine. No other pathology was seen. Microscopic examination of the viscera, heart, lungs, and muscle failed to show any histological changes.

In this study with the hydrochloride salt, 10 mg./Kg./day produced no symptoms when fed for fifty-three weeks; 20 mg./Kg./day produced occasional gastrointestinal signs when fed for forty-two and fifty-three weeks; 40 mg./Kg./day produced neurogenic and gastrointestinal signs throughout the study in the one animal fed the amount for forty-four weeks; 50 mg./Kg./day was relatively nontoxic to one animal which was fed the drug for forty-four weeks but produced neurogenic and gastrointestinal signs in another animal receiving the drug for four weeks; and 80 mg./Kg./day and all higher doses were lethal.

High doses, which produced death immediately or within a few days produced the above symptoms and in addition clonic convulsions which recurred at regular intervals and finally led to exhaustion and death. The gastrointestinal signs consisted of vomiting and diarrhea. Symptoms reported here resemble those described for diphenhydramine (17) except no hyperesthesia was seen following the administration of the drug. In some animals excessive salivation occurred and went on to become a conditioned reflex. In one animal, originally fed by stomach tube, the salivation stopped when the drug was given in a gelatin capsule.

Second Phase (Dihydrogen Citrate Used for this Study).—Eight dogs were prepared for this study by obtaining normal values on their weight, electrocardiogram, urinalysis, blood count, and blood chemistry. Following this the animals were grouped as follows: two to receive placebos, two to receive 10 mg./Kg., two to receive 20 mg./Kg. and two to receive 40 mg./Kg. All doses were in terms

of the base of phenyltoloxamine. All medications, in gelatin capsules, were given orally daily except Sunday for one year. The animals were weighed weekly and the doses for the following week adjusted to these weights. Weekly checks of blood counts, blood chemistry, and urinalysis were done. Electrocardiograms were made from time to time on the two dogs receiving the highest dose.

Although there was initially a slight weight loss in all groups of animals, this weight was regained in a few weeks and all animals ended the experiment with a greater weight than when they started. Blood counts, blood chemistry, and urinalysis in the test animals followed the same pattern as the controls and were within normal limits throughout. The electrocardiograms taken on the high dose dogs did not change during the experiment.

Absorption, Distribution, and Excretion.—Phenyltoloxamine in the form of its salts is readily absorbed from the gastrointestinal tract and peritoneal cavity; it is rapidly effective intravenously. This is shown by the specific blocking experiments already described. It is distributed rapidly and generally throughout the body as is shown by the early appearance of toxic signs described in the toxicity experiments when high doses were given. As yet, very little is known of its destruction, conjugation or excretion. That a detoxification mechanism involving degradation and/or excretion exists, is shown by the fact that large total doses are tolerated if the dose is administered by fixed fractions over a period of time. Attempts to isolate unchanged phenyltoloxamine or certain possible breakdown products from dogs' urine have been unsuccessful.

SUMMARY

1. Phenyltoloxamine (Bristamin), N,N-dimethyl-2-(α-phenyl-*o*-toloxy)-ethylamine, has been found to be a potent antagonist to many of the pharmacologic responses of histamine. Direct comparison of this material with diphenhydramine and tripelennamine have given favorable results.

2. Phenyltoloxamine has no undesirable effects at doses which are effective in combating the effects of histamine.

3. Acute toxicity studies indicate that phenyltoloxamine is considerably less toxic than tripelennamine and diphenhydramine. Chronic toxicity studies indicate that phenyltoloxamine is one of the least toxic of the antihistaminic agents. A dose of 20 mg./Kg. by rapid intravenous injection into the dog is tolerated but 30 mg./Kg. is fatal. Dogs tolerate phenyltoloxamine dihydrogen citrate in daily oral doses (calculated in terms of base) of 20 mg./Kg. and 40 mg./Kg. with no untoward effects. These dogs did not show any evidence of blood dyscrasia at any time during the experiment.

4. The method of detoxification and/or excretion is unknown.

5. In addition to its specific antihistaminic properties, phenyltoloxamine possesses some antispasmodic properties.

6. In common with many antihistaminic agents, phenyltoloxamine possesses distinct local anesthetic properties.

REFERENCES

(1) Ungar, G., Parrot, J. L., and Bovet, D., *Compt. rend. soc. biol.*, **124**, 445(1937).
(2) Bovet, D., and Staub, A. M., *ibid.*, **124**, 547(1937).
(3) Staub, A. M., *Ann. inst. Pasteur*, **63**, 485(1939).
(4) Cheney, L. C., Smith, R. R., and Binkley, S. B., *J. Am. Chem. Soc.*, **71**, 60(1949).
(5) Goodman, L., and Loewe, W. S., private communication.
(6) Mills, J., Rohrmann, E., Dinwiddie, W. G., and Lee, H. M., *Arch. intern. pharmacodynamie*, **80**, 119(1949).
(7) Hisamoto, K., *J. Pharm. Chem. Japan*, **24**, 127(1952); *C. A.*, **46**, 8226(1952).
(8) Hoople, G., and Brewer, D., to be published.
(9) Seyler, L. E., and Simon, S. W., *J. Allergy*, **24**, 261 (1953).
(10) Magnus, R., *Arch. ges. Physiol.*, **102**, 123(1904).
(11) Loew, E. R., Kaiser, Margaret E., and Moore, V., *J. Pharmacol. Exptl. Therap.*, **83**, 120(1945).
(12) SRG Report No. 255, "Sequential Analysis of Statistical Data," Applications Section 6, Columbia University Press, 1945.
(13) Jackson, D., "Experimental Pharmacology and Materia Medica," 2nd ed., The C. V. Mosby Co., St. Louis, Mo., 1939, p. 89.
(14) Barbour, H. G., *J. Pharmacol. Exptl. Therap.*, **7**, 547 (1915).
(15) Winter, C. A., *ibid.*, **94**, 7(1948).
(16) deBeer, E. J., *ibid.*, **85**, 1(1945).
(17) Gruzhit, O. M., and Fisher, R. A., *ibid.*, **89**, 227 (1947).

Antitubercular Substances. III. Nonpyridinoid Heterocyclic Hydrazides*

By ROBERT I. MELTZER, ARNOLD D. LEWIS, FREEMAN H. McMILLAN, JEROME D. GENZER, FREDERICK LEONARD, and JOHN A. KING

A series of twenty-four nonpyridinoid heterocyclic hydrazides representing ten different heterocyclic nuclei has been prepared and characterized. Although the majority of the substances prepared exhibited no useful *in vivo* antitubercular activity, some of them had an activity of the same order of magnitude as that of isonicotinic hydrazide.

As another aspect of our (1, 2) program directed toward synthetic antitubercular substances we are now describing the preparation of a number of hydrazides and some N^2-substituted hydrazides derived from heterocyclic carboxylic acids of other than the pyridine series.

Because the pyridyl-2- and -3-hydrazides were reported by Bernstein, *et al.* (3), and the -3-hydrazide subsequently by Bavin, *et al.* (4), to be relatively ineffective tuberculostatic compounds we did not attempt any further modifications in the pyridine series. In the diazine series, we reported in one of our earlier papers (1) that both 6-pyridazone-3-carboxhydrazide and 6-methyl-4,5-dihydro-3-pyridazone-4-carboxhydrazide were inactive; in view of the report by Rogers, *et al.* (5), that pyridazine-3-carboxamide is definitely antitubercular whereas the isomeric 4-carboxamide is inactive, one may conclude that either (*a*) the oxo function in the hetero ring invalidates any structure-activity relationships which were drawn from compounds containing nonoxygenated pyridazine rings, or (*b*) no relationship exists, either inverse (as in the pyridine series) or parallel, between ring-position and antitubercular activity of amides and hydrazides in the pyridazine or dihydropyridazine series. In the *meta*-diazine series, inactive pyrimidyl hydrazides have been reported by Bernstein and Yale, *et al.* (3, 6), and in the *para*-diazine series the 3-pyrazinoic hydrazide is likewise inactive (7); this latter report is of some interest in view of the activity reported by Birkofer (8) for the dibenzo *para*-diazine derivative, phenazine-1-carboxhydrazide, although it is true that in the phenazine derivative the hydrazide function is not directly attached to the heterocyclic ring. The reverse situation, in which the hetero-ring compound is active but its true benzo-derivative is relatively inactive, has been found in the quinoline series for cinchoninic hydrazide (3, 4) as well as for its 2-phenyl (4, and our No. 1), 2-(*p*-anisyl) (9), 2-(*p*-veratryl) (9), 3-hydroxy-2-phenyl (4), and 6-methoxy (4) derivatives, and for quinoline-2-carboxhydrazide (4)—all of which have a low order of activity.

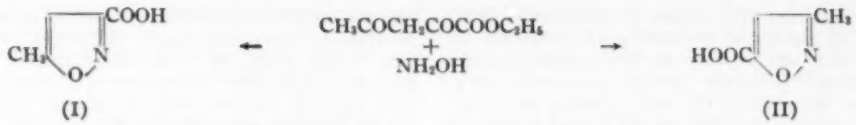
* Received February 27, 1953, from the Warner-Chilcott Research Laboratories, 113 West 18th Street, New York 11, N. Y.

The antitubercular evaluation of the substances described in this paper was carried out by Benjamin S. Schwartz, Lawrence Landis, and Dorothy Kley Crichlow, under the direction of Dr. Charles J. Duca, to all of whom we are greatly indebted. Compound No. 11 was prepared by Dr. John Shavel, Jr. Microanalyses were performed under the supervision of Dr. F. A. Buhler.

DISCUSSION

Since it appeared, therefore, that there was little hope of our finding any other highly active compounds in the six-membered nitrogenous heterocyclic series, we turned our attention to the five-membered nitrogenous heterocycles. We thus prepared the 2-pyrrolidyl compound (No. 2) and its 4,5-benzo derivative, indole-2-carboxhydrazide (No. 3), both of which were not active. Representatives of both the 1,2- and 1,3-diazoles were made, in pyrazole-3-carboxhydrazide (No. 4), and in imidazole-4-carboxhydrazide (No. 5), which also were both inactive; Buu-Hoi (9) has similarly found pyrazole-3-carboxhydrazide to be inactive and Yale, *et al.* (6), reported that a benzimidazole carboxhydrazide was without activity. The vicinal-triazole derivative (No. 6) also was inactive so we went no further in this series.

With the thought that an isoxazole with a carboxyl function in 5-position might be somewhat analogous to a pyridine with the carboxyl in the 4-position (i. e., the carboxyl is γ to the nitrogen in both) we prepared compound No. 7. There has been a certain lack of information in the literature with respect to the exact synthesis of the requisite acid. It has been reported (10–12) that when (eth)-oxalyl acetone is warmed on a water bath with hydroxylamine hydrochloride in acetic acid the ketone group adjacent to the carboxyl reacts first and the product is 5-methylisoxazole-3-carboxylic acid (I), m. p. 176°, while in Beilstein (13) it is stated that when the sodium enolate of ethoxalylacetone is allowed to stand with fuming hydrochloric acid, then treated with hydroxylamine hydrochloride and evaporated on the water bath after several days, one obtains 3-methylisoxazole-5-carboxylic acid (II), m. p. 211°; for the latter procedure there are given references to the paper by Claisen (11) and to a private communication from Claisen. In his paper (11) Claisen mentioned the acid II and said he would describe its preparation later, but apparently never did so. The acid II has been subsequently prepared by several Italian investigators (14–18), who also obtained by another route (15) an acid of m. p. 210–211° which was identical with that obtained by Claisen's method for II, and which could



(I) (II)

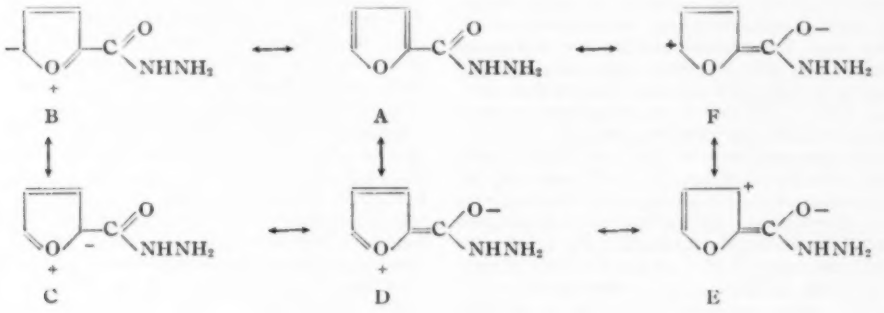
not have had structure I; therefore, the structure of II may be considered established. It is perhaps of interest that when we converted methoxalylacetone to a methylisoxazole carboxylic acid we presumably obtained both isomers I and II which were separated by fractional crystallization. Compound No. 7, the hydrazide of II, was without interest as an antitubercular substance.

While the isosteric thiazole and pyridine rings have both been found effective in sulfa drugs the same is apparently not true in antituberculars, a thiazole carboxylic hydrazide having been reported as inactive by Yale and co-workers (6). They also tested the hydrazide of 2-thienoic acid which we found (No. 8) to have some activity, but not of a useful nature; the thiophene derivatives (Nos. 9 and 10) with a methylene group interposed between the ring and hydrazide group were inactive.

The testing of furan derivatives for their antitubercular action has enjoyed some popularity for several years. Shpanir and Chertkova (19) studied fifteen amides of furan-2-carboxylic acid and reported the benzylamide to be active *in vitro* and *in vivo* but Smith, *et al.* (20), could not verify this. Long found (21) 5-nitro-2-furoamide to have little or no action. Thiosemicarbazones have been made of 2-furaldehyde (22–27), its 5-nitro- (24–29), 5-chloro- (24), and 5-hydroxymethyl- (30) derivatives as well as of 2-furylacrolein (26, 27) and of furoin, 2-acetylfuran, 2-butyrylfuran, and 2,5-dimethyl-3-acetylfuran (22), and have been tuberculostatically evaluated. Two furylacrylic acids were found (31) to be of no interest and of fifty-six furoamides, furylacrylic acids, and miscellaneous furan compounds tested (32), none was active *in vivo*. It was therefore of some interest that 2-furoic hydrazide (No. 11) displayed some activity *in vivo*, as has also been recently reported by Yale and co-workers (6), who also found activity in its N^1-dimethyl derivative, but not in its 3-methyl derivative or in the 3-furoic hydrazide. We found useful activity neither in the benzo derivative (No. 12) nor in the tetrahydro derivative (No. 13).

If the antitubercular action of 2-furoic hydrazide is in any integral manner associated with the maintenance of or shift in the contribution of any of the individual limiting structures, (A–F, *et al.*), whether this action is by copper-complex formation (33), by decreasing oxygen consumption and catalase activity (34), or the diamine oxidase and guanidine deamidase activity (35) of the tubercle bacilli, or by some as yet unsuggested mechanism, it should be possible to alter the activity of the molecule by the introduction of electron-withdrawing or -releasing groups at appropriate positions in the molecule.

Thus an electron-attracting group such as a nitro group in the 5-position (No. 14) might be expected to increase the contribution of form B, in which the carbonyl group does not participate in conjugated tautomeric displacement with the nuclear unsaturations; the same general situation, but to a much lesser extent, would be expected with the 5-hydroxymethyl derivative, No. 16; similarly, an electron-releasing group such as a chlorine atom in the 5-position (No. 15), would be expected to increase the contribution of form C (and the allied form in which the halogen atom carries the positive charge and is doubly-bonded to the 5-position), in which the carbonyl group also does not share in tautomeric interaction with the ring. An alkyl group, which can effect electron-release to the ring both inductively and hyperconjugatively, would favor the contribution of forms such as E (or the similar one in which there is an exocyclic double bond from the 3-position to the alkyl group) if it were in the 3-position (6) or of a form similar to B but with the positive charge in the 4-position (or the similar one in which there is an exocyclic double bond from the 4-position to the alkyl group) if it were in the 4-position (No. 17). It is perhaps significant that no nuclear substituent in 2-furoic hydrazide will permit of as great a contribution of extended conjugative tautomeric forms such as D, F, and the like to the structure of the molecule as is extant in the otherwise unsubstituted compound; it is to be noted that it is precisely these forms which are analogous to the extreme limiting structures which can be written for 4-pyridyl carboxhydrazide; whether these considerations are the explanation of the relative inactivity of all of the above noted nuclear-substituted furoic hydrazides, or whether they are merely coincidental cannot at present be stated.



The situation with respect to substitution in the hydrazide portion of the molecule is somewhat different and, just as with the hydrazine-substituted isonicotinic hydrazides previously described by us (2), the majority of the corresponding furoic hydrazide derivatives (Nos. 18–24) had an activity *in vivo* of the order of magnitude of that of the parent molecule.

All of the substances (Nos. 1–24) were evaluated for their action *in vitro* against *Mycobacterium tuberculosis*, strains $H_{37}R_V$ and D_4M_2, and by the drug-diet technique in mice infected with the virulent bovine D_4M_2 strain. Further details of the evaluation of the substances will be published elsewhere by the responsible investigators.

EXPERIMENTAL

2-Phenylcinchoninic Hydrazide, No. 1.—A mixture of ethyl 2-phenylcinchoninate (27.6 Gm., 0.10 mole) and 85% hydrazine hydrate (6.4 Gm., 0.13 mole) was heated under reflux for three hours. The crystals which separated from the cooled mixture were removed by filtration and washed with water. The product weighed 12.0 Gm. (45% yield) and melted at 228–229.5°. After recrystallization from 95% alcohol (800 cc.) it weighed 9.3 Gm. and was analytically pure.

Pyrazole-3-carboxylic Hydrazide, No. 4.—Methyl acrylate was treated with diazomethane and the resultant carbomethoxypyrazoline was oxidized with bromine according to the procedure of von Pechmann (42). Purification of the thus produced carbomethoxypyrazole was difficult so the material was converted to carboxypyrazole as described by von Pechmann and the purified acid was re-esterified by refluxing for several hours in ethanol saturated with dry hydrogen chloride. The carbethoxypyrazole obtained melted at 160°, as Knorr (37) reported, without indicating the source of his material. This product was heated on a steam bath for two hours with twice its weight of 85% hydrazine hydrate. Evaporation of the excess hydrazine left a residue which, after recrystallization from methanol, melted at 174–176° and was analytically pure, although another recrystallization raised the m. p. to 177.5–178°.

Imidazole-4-carboxhydrazide, No. 5.—Cupric acetate oxidation of fructose in the presence of ammonia and formaldehyde (43) gave 4(5)-hydroxymethylimidazole. The crude material, obtained by evaporation of the aqueous filtrate from removal of the copper as copper sulfide, was oxidized by means of nitric acid (44) to 4(5)-carboxyimidazole. An alcoholic solution of this acid was saturated with hydrogen chloride and refluxed for eight hours; evaporation of the ethanol and precipitation of the residue from its aqueous solution by potassium carbonate (to *p*H 11) gave 4(5)-carbethoxyimidazole, m. p. 153–158° [reported (44) for pure compound, 162°]. This ester was heated on a steam bath for one-half hour with 85% hydrazine hydrate; the hydrazide obtained by filtration of the cooled reaction mixture melted at 203–204° [reported (38) for pure compound, 210°] after it was washed with water; after recrystallization from water it was pure.

1,2,3-Triazole-4-carboxhydrazide, No. 6.—A solution of hydrazoic acid (45), prepared from 0.20 mole sodium azide, in 80 cc. of ether was added to 11.4 Gm. (0.17 mole) propiolic acid in 100 cc. of ether and the mixture was allowed to stand at room temperature for forty-eight hours. Filtration gave 2.3 Gm. (12% yield) of *v*-triazole-4-carboxylic acid (46), m. p. 210–212°. A mixture of 1.5 Gm. of the acid, 1.8 Gm. ethanol, 0.25 cc. sulfuric acid, and 5.5 cc. ethylene dichloride was heated under reflux on the steam bath for three hours, after which time it was a homogeneous solution. The precipitate resulting from the addition to the solution of 2.4 Gm. barium carbonate was removed by filtration and the 4-carbethoxy-*v*-triazole obtained as a residue on evaporation of the solvent from the filtrate was heated on the steam bath for several hours with 2 cc. 85% hydrazine hydrate. The hydrazide obtained as a residue on evaporation of the excess hydrazine melted at 252° (dec.) after recrystallization from water.

3-Methylisoxazole-5-carboxhydrazide, No. 7.—A solution of methyl acetopyruvate (47) (33 Gm., 0.23 mole) and hydroxylamine hydrochloride (16.0 Gm., 0.23 mole) in water (125 cc.) was saturated with hydrogen chloride at ice-bath temperature and was then stoppered and stored in a refrigerator for four days. The reaction mixture, which now contained a precipitate, was poured into ice water and then filtered to give 16.9 Gm. (58% yield) of crude methylisoxazole carboxylic acid, m. p. 201–203° (dec.). This material was dissolved in 135 cc. acetone, then the filtered solution was diluted with 90 cc. petroleum ether (b. p. 60–68°) and allowed to crystallize first at room temperature and then in the refrigerator. The precipitate of pure 3-methylisoxazole-5-carboxylic acid weighed 9.25 Gm. (32% yield) and melted at 211.5° (dec.) (13). Evaporation under vacuum of the mother liquor from the crystallization of this acid furnished 6.7 Gm. of acid, m. p. 170–177°, that was presumed to be the isomeric 5-methylisoxazole-3-carboxylic acid, reported (10–12) to melt at 176°, which was not further investigated. The 3-methylisoxazole-5-carboxylic acid was converted to 5-carbethoxy-3-methylisoxazole (48), m. p. 28°, by refluxing ethanolic sulfuric acid. A mixture of 5-carbethoxy-3-methylisoxazole (11.3 Gm., 0.073 mole) and 85% hydrazine hydrate (4.3 Gm., 0.073 mole) spontaneously warmed and was thereafter allowed to stand at room temperature for four hours. The resultant crystals, after filtration and washing with ether, weighed 7.7 Gm. (75% yield) and melted at 135–137°. After recrystallization from 60 cc. ethanol the pure hydrazide weighed 6.2 Gm.

2-Thienylacethydrazide, No. 9.—A mixture of ethyl 2-thienylacetate (49) (51 Gm., 0.30 mole) and 85% hydrazine hydrate (22.5 Gm., 0.38 mole) was heated on the steam bath for two hours and then cooled. The resultant precipitate, which was removed by filtration, washed with water and then dried, weighed 15.5 Gm. and melted at 99–100°. After recrystallization from 150 cc. benzene the analytically pure product weighed 14.3 Gm.

5-Chloro-2-thienylacethydrazide, No. 10.—A mixture of ethyl 5-chloro-2-thienylacetate (50) (44.7 Gm., 0.22 mole) and 85% hydrazine hydrate (15.5 Gm., 0.26 mole) was heated on a steam bath for four hours and then allowed to stand overnight at room temperature. The crystalline product was removed by filtration and washed with a little water; after being dried, it weighed 34 Gm. (81% yield) and melted at 103–104°. After recrystallization from

300 cc. of benzene the material weighed 31 Gm. and was analytically pure.

Tetrahydro-2-furoic Hydrazide, No. 13.—A mixture of ethyl tetrahydro-2-furoate (51) (14.4 Gm., 0.10 mole), 85% hydrazine hydrate (6.5 Gm., 0.13 mole) and ethanol (100 cc.) was refluxed for one hour and then fractionally distilled to give the product, which after a second distillation boiled at 112–113° (2 mm.) and weighed 3.0 Gm. (23% yield).

5-Nitro-2-furoic Hydrazide, No. 14.—A solution of methyl 5-nitro-2-furoate (purchased from Eaton Laboratories, Inc., Norwich, N. Y.) (10 Gm., 0.058 mole) and 85% hydrazine hydrate (3.5 Gm , 0.070 mole) in methanol (300 cc.) was allowed to stand at room temperature for several hours, during which time golden crystals of the hydrazide precipitated. After recrystallization from methanol the analytically pure product weighed 3.0 Gm. (30% yield).

5-Chloro-2-furoic Hydrazide, No. 15.—Ethyl 5-chloro-2-furoate (52) (20.4 Gm., 0.117 mole) and 100% hydrazine hydrate (60 cc.) were heated on the steam bath for three hours. Evaporation of the excess hydrazine left a tacky semisolid which was extracted three times with 100 cc.-portions of hot benzene. Concentration and chilling of the combined benzene extracts gave the hydrazide which, after recrystallization from benzene, weighed 4.9 Gm. (24% yield) and was analytically pure.

5-Hydroxymethyl-2-furoic Hydrazide, No. 16.—A mixture of ethyl 5-hydroxymethyl-2-furoate (53) (4.6 Gm., 0.027 mole) and 100% hydrazine hydrate (15 cc., 0.309 moles) was heated on the steam bath for two hours and then concentrated under 15 mm. pressure on the steam bath to leave a residue that solidified on cooling. After two recrystallizations from ethanol, the analytically pure product weighed 2.8 Gm. (74% yield).

4-Isopropyl-2-furoic Hydrazide, No. 17.—4-Isopropyl-2-furoic acid (54, 55) (12.1 Gm., 0.078 mole) in 120 cc. ethanol containing 5% by weight of dry hydrogen chloride was refluxed for seven hours. The solvent was then removed under vacuum, the residue taken up in 150 cc. dry ether and the solution shaken with anhydrous sodium carbonate in order to neutralize any remaining organic or inorganic acid. After filtration of the sodium carbonate the solvent was evaporated from the filtrate and the residue was distilled to give 11.7 Gm. (82% yield) of ethyl 4-isopropyl-2-furoate, b. p. 122–123° (14 mm.), n_D^{25} 1.4744.

Anal.—Calcd. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 66.25; H, 8.03.

The above ester (11.7 Gm., 0.064 mole) was refluxed for three hours with 35 cc. (0.7 mole) of 100% hydrazine hydrate, then the excess hydrazine was removed on the steam bath at 15 mm. pressure. The residue, which solidified on cooling, was recrystallized twice from 1:3 ethanol-water to give 7.4 Gm. (68% yield) of pure hydrazide.

N^1-(2-Furoyl)-N^2-(4-aminobenzenesulfonyl)-hydrazine, No. 18.—A mixture of 2-furoic hydrazide (3.12 Gm., 0.025 mole), 4-acetamidobenzenesulfonyl chloride (9.10 Gm., 0.037 mole), and 50 cc. of 10% sodium hydroxide solution was shaken vigorously with external cooling until an homogeneous solution resulted. The solution was then acidified and the precipitated solid was recrystallized from aqueous alcohol to give 7.0 Gm. (94% yield) of N^1-(2-furoyl)-N^2-(4-acetamidobenzenesulfonyl)-hydrazine, m. p. 215–217°. Four Gm. (0.0124 mole) of this acetamido compound was refluxed with 18 cc. of 4:1 aqueous hydrochloric acid until a clear solution was obtained. This solution was diluted with water and made basic with sodium carbonate; the precipitated solid was recrystallized from ethanol to give 2.0 Gm. (57% yield) of product, m. p. 203.5–205°, which after another recrystallization was analytically pure.

N^1-(2-Furoyl)-N^2-(1-methoxy-2-propylidene)-hydrazine, No. 19.—A solution of methoxyacetone (56) (56.3 Gm., 0.64 mole) in 300 cc. benzene was refluxed a short while under a Dean and Starke water trap to remove any traces of moisture present, then furoic hydrazide (25.2 Gm., 0.20 mole) was added and refluxing was continued until the theoretical amount of water had been collected (one hour). The solvent and excess ketone were removed, first on the water pump and then with a vacuum pump to leave a residue which crystallized on cooling and scratching. This residue was charcoaled in carbon tetrachloride, petroleum ether (b. p. 60–68°) was added to incipient cloudiness, and the product crystallized from the slowly cooled solution. It weighed 15.7 Gm. (40% yield) and was pure after another recrystallization.

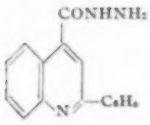
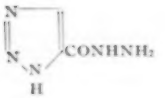
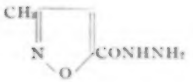
N^1-(2-Furoyl)-N^2-(1-methoxy-2-propyl)-hydrazine, No. 20.—N^1-(2-Furoyl)-N^2-(1-methoxy-2-propylidene)-hydrazine (5.6 Gm., 0.028 mole) was dissolved in a suspension of 0.2 Gm. prereduced Adams platinum oxide in 150 cc. dry ethanol and the mixture was hydrogenated at room temperature on a Parr shaker, using a gas burette. The reduction was complete after three hours (theoretical hydrogen uptake). The reduction mixture was worked up in the usual manner to give the product, b. p. 153–163° (0.6 mm.), n_D^{26} 1.520, which could not be induced to crystallize.

N^1-(2-Furoyl)-N^2-(4-carbethoxy-2-butylidene)-hydrazine, No. 21.—A mixture of furoic hydrazide (25.2 Gm., 0.20 mole) and ethyl levulinate (43.3 Gm., 0.30 mole) in 130 cc. absolute ethanol was refluxed one hour and the solvent then stripped off under vacuum. The residue, which slowly crystallized, was washed with ether and then recrystallized from carbon tetrachloride to give 48 Gm. (95% yield) of pure product.

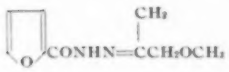
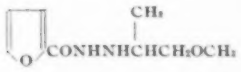
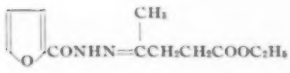
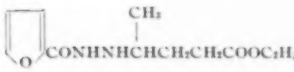
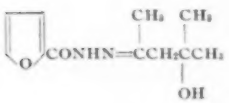
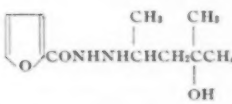
N^1-(2-Furoyl)-N^2-(4-carbethoxy-2-butyl)-hydrazine, No. 22.—A solution of 10.19 Gm. (0.040 mole) of N^1-(2-furoyl)-N^2-(4-carbethoxy-2-butylidene)hydrazine in 140 cc. absolute ethanol containing 0.3 Gm. of suspended Adams platinum oxide which had been prereduced at room temperature was hydrogenated on a Parr shaker at room temperature for four and one-half hours, at the end of which time the theoretical amount of hydrogen had been taken up. After filtration of the catalyst the solvent was removed from the filtrate to leave a residue which, after three recrystallizations from carbon tetrachloride, was analytically pure.

N^1-(2-Furoyl)-N^2-(4-hydroxy-4-methyl-2-pentylidene)-hydrazine, No. 23.—A mixture of furoic hydrazide (25.2 Gm., 0.20 mole) and diacetone alcohol (46.4 Gm., 0.40 mole) was gently warmed on the steam bath until an exothermic reaction occurred that increased the internal temperature to 95° and then let it slowly fall to 91°. Heating was then continued for one hour and the mixture was chilled. The resultant crystals were filtered and recrystallized from isopropanol to give 16 Gm. (35% yield) of pure product.

NONPYRIDINOID HETEROCYCLIC HYDRAZIDES

No.		M. P., °C.	Empirical Formula	Analyses—Calculated C	Calculated H	Found C	Found H
1.		230 (dec.)	$C_{14}H_{13}N_3O$[a]	72.98	4.97	73.43	4.63
2.		228 (dec.)	(36)				
3.		247–248	(36)				
4.		174–175[b]	$C_4H_6N_4O$	38.09	4.80	37.93	4.72
5.		203–204 (dec.)	(38)				
6.		252–253 (dec.)	$C_3H_5N_5O$	28.35	3.97	28.43	4.11[c]
7.		136–137	$C_5H_7N_3O_2$	42.55	5.00	42.80	5.14
8.		134–136	(39)				
9.		100	$C_6H_9N_3OS$	46.13	5.16	46.38	5.41[d]
10.		104–105	$C_6H_7ClN_2OS$	37.79	3.70	37.94	3.60
11.		76–78	(40)				
12.		172–173	(41)				
13.		b. p. 112–113 (2 mm.)	$C_6H_{10}N_2O_2$	46.14	7.74	45.66	7.68
14.		173–174	$C_5H_5N_3O_4$	35.09	2.95	35.36	3.18

(Continued on next page)

15.		124–125	$C_6H_5ClN_2O_2$	37.40	3.14	37.45	3.33[e]
16.		140	$C_6H_8N_2O_3$	46.15	5.16	46.42	5.36[f]
17.		85–86	$C_8H_{12}N_2O_2$	57.12	7.19	57.13	7.54[g]
18.		205–206	$C_{11}H_{11}N_3O_4S$	46.97	3.94	46.99	4.13
19.		91–92	$C_9H_{12}N_2O_3$	55.10	6.17	54.95	6.45[h]
20.		b. p. 153–163 (0.6 mm.)	$C_9H_{14}N_2O_3$	54.53	7.12	54.82	6.74
21.		91–92	$C_{12}H_{18}N_2O_4$	57.13	6.39	57.07	6.09[i]
22.		86–87	$C_{12}H_{18}N_2O_4$	56.67	7.13	56.92	6.86
23.		131–132	$C_{11}H_{16}N_2O_3$	58.91	7.19	59.18	6.96[j]
24.		114–116	$C_{11}H_{16}N_2O_3$	58.39	8.02	58.10	7.83

[a] The *in vitro* activity of this substance was reported by Bavin and co-workers (4) but no characterization of the compound is in the chemical literature.

[b] This compound, m. p. 174–176°, was reported by Knorr (37), but neither its preparation nor its analysis was given; Buu-Hoi and co-workers (9) reported on its activity but did not describe its preparation.

[c] Nitrogen: Calcd. 55.95; Found: 55.10.
[d] Nitrogen: Calcd. 17.94; Found: 17.67.
[e] Nitrogen: Calcd. 17.45; Found: 17.59.
[f] Nitrogen: Calcd. 17.95; Found: 18.22.
[g] Nitrogen: Calcd. 16.66; Found: 17.00.
[h] Nitrogen: Calcd. 14.28; Found: 14.27.
[i] Nitrogen: Calcd. 11.11; Found: 11.25.
[j] Nitrogen: Calcd. 12.39; Found: 12.51.

N^1-(2-Furoyl)-N^2-(4-hydroxy-4-methyl-2-butyl)-hydrazine, No. 24.—A solution of 4.5 Gm. of the corresponding hydrazone was hydrogenated by a method analogous to the one used above. The product was recrystallized from isopropanol.

REFERENCES

(1) Shavel, J., Leonard, F., McMillan, F. H., and King, J. A., THIS JOURNAL, **42**, 402(1953).
(2) McMillan, F. H., Leonard, F., Meltzer, R. I., and King, J. A., *ibid.*, **42**, 457(1953).
(3) Bernstein, J., Lott, W. A., Steinberg, B. A., and Yale, H. L., *Am. Rev. Tuberc.*, **65**, 357(1952).
(4) Bavin, E. M., Drain, D. J., Seiler, M., and Seymour, D. E., *J. Pharm. Pharmacol.*, **4**, 844(1952).
(5) Rogers, E. F., Leanza, W. J., Becker, H. J., Matzuk, A. R., O'Neill, R. C., Basso, A. J., Stein, G. A., Solotorovsky, M., Gregory, F. J., and Pfister, K., *Science*, **116**, 253(1952).
(6) Yale, H. L., Losee, K., Martins, J., Holsing, M., Perry, F., and Bernstein, J., Paper No. 25 presented before the Division of Medicinal Chemistry at the 122nd American Chemical Society meeting, Atlantic City, N. J., September 17, 1952.
(7) Kushner, S., Dalalian, H., Sanjurjo, J. L., Bach, F. L., Safir, S. R., Smith, V. K., and Williams, J. H., *J. Am. Chem. Soc.*, **74**, 3617(1952).
(8) Birkofer, L., *Angew. Chem.*, **64**, 111(1952).
(9) Buu-Hoi, N. P., Welsch, M., Dechamps, G., Le Bihan, H., Binon, F., and Mentzer, C., *Compt. rend.*, **234**, 1925(1952).
(10) Claisen, L., *Ber.*, **24**, 3900(1891).
(11) Claisen, L., *ibid.*, **42**, 59(1909).
(12) Mumm, O., and Bergell, C., *ibid.*, **45**, 3040(1912).
(13) Beilstein's "Handbuch der Organischen Chemie," 4th ed., Vol. XXVII, 1937 p. 315.
(14) Quilico, A., and Panizzi, L., *Gazz. chim. ital.*, **68**, 625(1938).
(15) Quilico, A., and Panizzi, L., *ibid.*, **69**, 536(1939).
(16) Musante, C., *ibid.*, **72**, 242(1942).
(17) Panizzi, L., *ibid.*, **73**, 99(1943).
(18) Quilico, A., Fusco, R., and Rosnati, V., *ibid.*, **76**, 87 (1946).
(19) Shpanir, F. L., and Chertkova, E. I., "Problems of Tuberculosis," **1944**, No. 4, 9.
(20) Smith, M. I., Bauer, H., Chang, Y. T., and Fried-

lander, H., *Proc. Soc. Exptl. Biol. Med.*, **73**, 563(1950).
(21) Summary Tables of Biological Tests (Chemical-Biological Coordination Center, National Research Council, Washington 25, D. C.), **4**, 338(1952).
(22) Duca, C. J., Rothlauf, M. V., and Scudi, J. V., *Antibiotics and Chemotherapy*, **2**, 16(1952).
(23) Bernstein, J., Yale, H. L., Losee, K., Holsing, M., Martins, J., and Lott, W. A., *J. Am. Chem. Soc.*, **73**, 906 (1951).
(24) Hagenbach, R. E., and Gysin, H., *Experientia*, **8**, 184 (1952).
(25) Hamre, D., Bernstein, J., and Donovick, R., *J. Bact.*, **59**, 675(1950).
(26) Sah, P. P. T., and Daniels, T. C., *Rec. trav. chim.*, **69**, 1545(1950).
(27) Donovick, R., Pansy, F., Stryker, G., and Bernstein, J., *J. Bact.*, **59**, 667(1950).
(28) Hoggarth, E., Martin, A. R., Storey, N. E., and Young, E. H. P., *Brit. J. Pharmacol.*, **4**, 248(1949).
(29) Stillman, W. B., and Scott, A. B., U. S. pat. 2,416,239, February 18, 1947.
(30) Gardner, T. S., Smith, F. A., Wenis, E., and Lee, J., *J. Org. Chem.*, **16**, 1121(1951).
(31) Naito, M., Take, T., Shioda, A., Fujikawa, F., Nakajima, K., Fujii, H., Tokuoka, A., Okamoto, M., and Hitosa, Y., *J. Pharm. Soc. Japan*, **71**, 811(1951); *Chem. Abstr.*, **46**, 1615(1952).
(32) Freedlander, B. L., and Furst, A., Wasmann *J. Biol.*, **9**, 355(1951).
(33) Fallab, S., and Erlenmeyer, H., *Experientia*, **8**, 298 (1952).
(34) Aronson, J. D., Ehrlich, S. L., and Flagg, W., *Proc. Soc. Exptl. Biol. Med.*, **80**, 259(1952).
(35) Zeller, E. A., Barsky, J., Fouts, J. R., Kirchheimer, W. F., and Van Orden, L. S., *Experientia*, **8**, 349(1952).
(36) Piccinini, A., and Salmoni, L., *Gazz. chim. ital.*, **32I**, 246(1902).
(37) Knorr, L., *Ber.*, **37**, 3520(1904).
(38) Balaban, I., *J. Chem. Soc.*, **1930**, 268.
(39) Curtius, T., and Thyssen, H., *J. prakt. Chem.*, [2], **65**, 1(1902).
(40) Curtius, T., and Leimbach, R., *ibid.*, **65**, 20(1902).
(41) Stoermer, R., and Calov, G., *Ber.*, **34**, 770(1901).
(42) von Pechmann, H., and Burkard, E., *Ber.*, **33**, 3594 (1900).
(43) Totter, J. R., and Darby, W. J., "Organic Syntheses," John Wiley & Sons, Inc., New York, N. Y., Vol. XXIV, 1944, p. 64.
(44) Pyman, F. L., *J. Chem. Soc.*, **109**, 186(1916).
(45) Wolff, H., "Organic Reactions," John Wiley & Sons, Inc., New York, N. Y., Vol. III, 1946, p. 327.
(46) Oliveri-Mandala, E., and Coppola, A., *Gazz. chim. ital.*, **40II**, 435(1910).
(47) Royals, E. E., *J. Am. Chem. Soc.*, **67**, 1508(1945).
(48) Musante, C., *Gazz. chim. ital.*, **70**, 685(1940).
(49) Leonard, F., *J. Am. Chem. Soc.*, **74**, 2915(1952).
(50) Cairns, T. L., and McKusick, B. C., *J. Org. Chem.*, **15**, 790(1950).
(51) Paul, R., and Hilly, G., *Compt. rend.*, **208**, 359 (1939).
(52) Hill, H. B., and Jackson, L. L., *Am. Chem. J.*, **12**, 112(1890).
(53) Haworth, W. N., and Jones, W. G. M., *J. Chem. Soc.*, **1944**, 667.
(54) Gilman, H., and Calloway, N. O., *J. Am. Chem. Soc.*, **55**, 4197(1933).
(55) Gilman, H., Calloway, N. O., and Burtner, R. R., *ibid.*, **57**, 906(1935).
(56) Mariella, R. P., and Leech, J. L., *ibid.*, **71**, 3558 1949).

The Effect of Antipyretics on the Erythrocytic Sedimentation Rate of Rats*

By DUANE G. WENZEL, JACK L. BEAL,† and ALBERT R. HASKELL‡

It has been demonstrated that even excessive doses of three different types of antipyretics (salicylates, pyrazalones, and aniline derivatives) do not significantly affect the normal erythrocytic sedimentation rate of rats. This might be regarded as evidence for the reliability of the normal sedimentation rate obtained while administering the antipyretics.

THE RATE of erythrocytic sedimentation is a valuable diagnostic test for several pathological conditions. Cancer (1, 2), tuberculosis (3), rheumatic fever (4), and various acute inflammatory diseases produce an increased speed of sedimentation. This change is also observed after the third or fourth month of pregnancy (5).

Although the cause of the phenomenon is not clear, it is in some manner related to the protein components of the plasma (6). The effect has been duplicated *in vitro* by the addition of large, highly asymmetrical molecules such as acacia (7) and pneumococcus polysaccharide (8). *In vivo* experiments have demonstrated the rate to be reduced by the salicylates (2, 4, 9–11). This observation is of clinical significance as the salicylates are commonly self-administered and would thus interfere with the value of the sedimentation rate as a diagnostic aid.

A survey of the literature has revealed that the preceding observations on the salicylates were made with pathological conditions. The purpose of this study is to determine the effects of salicylates and two other common chemical types of antipyretics, the pyrazalone and aniline derivatives, on the normal sedimentation rate. As examples of each type, acetylsalicylic acid, aminopyrine, and acetophenetidin are tested. Rats, rather than humans, are used in the experiment because large doses with possibly

* Received March 25, 1953, from the School of Pharmacy, University of Kansas, Lawrence.
† Assistant Professor of Pharmacognosy, College of Pharmacy, Ohio State University, Columbus, Ohio.
‡ Instructor in Pharmacology, College of Pharmacy, University of Florida, Gainesville, Florida.

TABLE I.—MEAN ERYTHROCYTIC SEDIMENTATION RATES OF RATS ADMINISTERED ANTIPYRETICS

Drug	Drug in Food, %	Mean Weekly Sedimentation Rates, in Mm./Hr. ± S. E.[a]			
		1	2	3	4
Controls	0	1.61 ± 0.19	1.45 ± 0.17	1.42 ± 0.10	1.58 ± 0.14
Acetylsalicylic acid	0.1	1.47 ± 0.29	1.15 ± 0.13	1.37 ± 0.26	1.25 ± 0.12
	0.33	1.53 ± 0.13	1.91 ± 0.07	1.91 ± 0.11	1.70 ± 0.13
	0.66	1.59 ± 0.19	1.71 ± 0.18	1.57 ± 0.11	1.85 ± 0.13
Acetophenetidin	0.1	2.21 ± 0.13	2.11 ± 0.16	1.75 ± 0.11	1.92 ± 0.10
	0.33	1.85 ± 0.08	1.70 ± 0.15	1.90 ± 0.10	1.87 ± 0.07
	0.66	2.18 ± 0.08	2.20 ± 0.24	1.90 ± 0.08	1.87 ± 0.08
Aminopyrine	0.1	1.71 ± 0.18	1.86 ± 0.10	1.93 ± 0.11	2.25 ± 0.13
	0.33	1.72 ± 0.13	1.90 ± 0.11	1.83 ± 0.08	1.84 ± 0.12
	0.66	2.08 ± 0.10	1.82 ± 0.10	1.86 ± 0.05	1.89 ± 0.03

[a] S. E. $= \sqrt{\frac{\Sigma Y^2 - \bar{Y}T}{N(N-1)}}$

dangerous sequelae can be administered and because conditions and subjects can be held to a greater degree of uniformity.

EXPERIMENTAL

The rats used were Wistar male albinos ranging in weight from 147 to 187 Gm. Groups of 8 rats were used for each drug at each of the three dosage levels and 8 as a control. The diet consisted of Purina Laboratory Chow to which was added 0.10, 0.33 or 0.66% of the drug. These drugs were dissolved in sufficient alcohol to moisten the food and the whole dried in a steam oven. The rats were given food and water *ad libitum*. Each animal consumed approximately 10 Gm. of food daily, equivalent to daily doses of 60, 198, and 396 mg./Kg.

The sedimentation rate was determined before administering the drugs and at weekly intervals thereafter for three weeks using the Cutler method (12). Cutler's test has as its object the determination of the sedimentation rate at a time when the rouleaux are formed and settling with maximum velocity. Results are expressed in mm. and there is no need for correction of the degree of anemia present.

One-tenth cubic centimeter of 3.8% sodium citrate was placed in a Cutler tube. One cubic centimeter of blood was withdrawn by cardiac puncture into a 2-cc. syringe and transferred from the syringe to the tube. The tube was inverted several times to allow complete mixture of the sodium citrate and blood. A cotton roll was used to absorb the excess blood, thus allowing the blood to rest exactly at the zero mark in the tube. The tubes were placed in a special rack which kept them absolutely vertical. The position of the sedimenting column of erythrocytes was determined at the end of one hour.

RESULTS

As indicated by Table I, the sedimentation rates of all test groups and the controls do not show any consistent changes. Although a direct relationship cannot be established between the rat and human doses of these drugs, the range of 60 to 396 mg./Kg./day far exceeds doses encountered in human administration. The evidence thus emphasizes the difficulty of affecting a normal sedimentation rate by the usual doses of the antipyretics.

SUMMARY

1. Antipyretics of three distinct chemical types are shown to have no consistent effect upon the normal erythrocytic sedimentation rate of rats.

2. The results re-emphasize the difference in the effect of drugs on normal and pathological blood values.

REFERENCES

(1) Meyer, C., Hahnel, E., and Feiner, R., *Proc. Soc. Exptl. Biol. Med.*, **58**, 36(1945).
(2) Homburger, F., *Am. J. Med. Sci.*, **211**, 346(1946).
(3) Banyai, A. L., and Caldwell, E., *Am. Rev. Tuberc.*, **38**, 491(1938).
(4) Rapoport, S., and Guest, G. M., *Proc. Soc. Exptl. Biol. Med.*, **61**, 43(1946).
(5) Bland, P. B., Goldstein, L., and First, A., *Surg., Gynecol. Obstet.*, **50**, 429(1930).
(6) Nichols, R. E., *J. Lab. Clin. Med.*, **27**, 1317(1942).
(7) Fahraeus, R., *Acta Med. Scand.*, **55**, 3(1921).
(8) Nungester, W. J., and Klein, L. F., *Proc. Soc. Exptl. Biol. Med.*, **36**, 315(1937).
(9) Lichty, J. S., Jr., and Hooker, S. P., *ibid.*, **48**, 69 (1941).
(10) Homburger, F., *Am. J. Med. Sci.*, **210**, 168(1945).
(11) Harris, T. N., *ibid.*, **213**, 482(1947).
(12) Cutler, J. W., *J. Lab. Clin. Med.*, **26**, 542(1940).

Oleanolic Acid from Cranberries*

By BETTY Y. T. WU† and LLOYD M. PARKS

A modified procedure, involving decolorization of the ammonium salt, is described for the purification of crude ursolic acid obtained from cranberries. Oleanolic acid was isolated from the mother liquors remaining from the final recrystallization of the purified product.

IN THEIR REPORT of the isolation of ursolic acid from cranberry pomace, *Vaccinium macrocarpum* Ait., Markley and Sando (1) noted the presence of another unidentified resin acid in the ether-soluble fraction. In the course of purification of crude ursolic acid,[1] obtained by extraction of cranberries, we have isolated and identified oleanolic acid, whose presence in that plant has not heretofore been reported.

Kremers, *et al.* (2), using a procedure based on the fact that sodium ursolate is only slightly soluble in aqueous alkali while most of the accompanying impurities are soluble in this solvent, reported better success in the purification of crude ursolic acid than by use of the methods of Dodge (3) and Sando (4). Considerable difficulty was encountered by us with the Kremers procedure in decolorizing the deeply colored sodium ursolate and a yield of only 15 per cent of pure ursolic acid (based on weight of the crude) was obtained. In a modified procedure ammonium ursolate, prepared by saturation of a methanolic solution of crude ursolic acid, was decolorized with Norit and the free ursolic acid obtained by acidification. In this way yields of 22 to 30 per cent of pure ursolic acid were obtained with much less manipulation. Oleanolic acid was isolated from the mother liquors remaining from crystallization of the purified ursolic acid.

EXPERIMENTAL

Purification of Ursolic Acid by the Modified Procedure.—Three hundred grams of crude ursolic acid was suspended in 10 L. of methanol and a stream of ammonia gas was passed into it. At the end of one hour most of the solid had dissolved and filtration of the warm mixture through a cloth left only 20 Gm. of insolubles, which consisted chiefly of fibrous plant material and dirt.

The dark brown filtrate was boiled for one and one-half hours with 80 Gm. of Norit with vigorous stirring. After allowing to stand overnight the mixture was heated to boiling and filtered while hot through a 2 cm. layer of filter cel. Treatment of the filtrate twice more with Norit yielded a pale yellow colored solution. This solution was concentrated to about 3 L. to the appearance of a white precipitate, acidified with dilute hydrochloric acid and diluted with water to 6 L. The free ursolic acid which was precipitated was filtered by suction and washed well with water.

The semidried ursolic acid was dispersed in 4 L. of water, to the suspension was added a solution of 20 Gm. of sodium hydroxide in water, and the mixture was heated with stirring at its boiling point for one hour. The white insoluble sodium ursolate was then separated by centrifuging, washed with hot water, dissolved in 1½ L. of methanol and the solution acidified with dilute hydrochloric acid. The precipitated ursolic acid was filtered by suction, washed with water until free from chloride, and dried for twenty-four hours at 85°; it weighed 148 Gm., m. p. 270–276°.[2]

This semipure product was suspended in 2 L. of ethanol-acetone (3:1) and the mixture heated at its boiling point, with most of the ursolic acid remaining insoluble and settling to the bottom of the container. The hot saturated supernatant liquid was separated by decantation and immediately upon cooling small white needle crystals of ursolic acid formed. The crystalline product was filtered by suction, the mother liquor from this crystal crop was diluted with 200 ml. of fresh solvent and used to treat the insoluble residue of ursolic acid in the same manner as before. By repetition of this treatment several fractions of crystalline product were obtained as follows:

Fraction	Weight, Gm.	M. P., °C.
1	26.40	281–282
2	17.35	280–281.5
3	21.30	280–282
4	23.20	279–282
5	27.10	250–260
6	25.00	249–255

Fractions 1 and 2 were combined and after two recrystallizations from acetone-alcohol yielded 40.4 Gm. of white needles, m. p. 283–283.5°. Fractions 3 and 4 after four recrystallizations yielded 32.3 Gm. of the same product, m. p. 282.5–283.5°. No attempt was made at further purification of Fractions 5 and 6.

Additional purification runs on the original crude ursolic acid by the procedure described gave the pure crystalline acid in yields of 22, 22, and 30%, respectively.

Isolation of Oleanolic Acid.—Concentration of the mother liquors from the final recrystallizations of Fractions 3 and 4, above, to a volume of 30 ml. resulted in the separation of 11 Gm. of yellow crystal-

* Received March 16, 1953, from the School of Pharmacy, University of Wisconsin, Madison.
Supported in part by a research grant from the National Cranberry Association, Hanson, Massachusetts.
† National Cranberry Association Fellow in Pharmaceutical Chemistry, 1951–52.
[1] Kindly supplied by the National Cranberry Association.
[2] All melting points were obtained with Anschutz total immersion thermometers.

line powder. This was acetylated in the usual manner with acetic anhydride and anhydrous sodium acetate, the solid reaction product dissolved by refluxing with 300 ml. of 70% alcohol, and the resulting solution diluted with 500 ml. of water. The acetate separated as a white precipitate and was crystallized from absolute methanol to yield 4.25 Gm. of long white needles, m. p. 266–268°, which showed no depression (267–269°) when mixed with an authentic sample of acetyl oleanolic acid (5); $[\alpha]_D^{25°}$ = +75.4° (chloroform, C = 1.366).

One gram of the acetate was hydrolyzed by refluxing with alcoholic potassium hydroxide, the hydrolysis mixture acidified with diluted hydrochloric acid, diluted with water, and the white precipitate shaken out with several portions of ether. Evaporation of the ether solution left a white residue which was crystallized from absolute methanol to yield 0.65 Gm. of white needles of oleanolic acid, m. p. 312–314°, which showed no depression (311–313°) when mixed with an authentic sample (5). $[\alpha]_D^{25°}$ = +80.2° (chloroform, C = 0.988).

REFERENCES

(1) Markley, K. S., and Sando, C. E., *J. Biol. Chem.*, **105**, 643(1934).
(2) Kremers, R. E., Sell, H. M., and Stookey, A. D., unpublished data.
(3) Dodge, F. D., *J. Am. Chem. Soc.*, **40**, 1932(1918).
(4) Sando, C. E., *J. Biol. Chem.*, **90**, 477(1931).
(5) Rowe, E. J., *et al.*, THIS JOURNAL, **38**, 122(1949).

Chemical Studies on Ursolic Acid*

By BETTY Y. T. WU† and LLOYD M. PARKS

From ursolic acid, $C_{29}H_{46}\begin{cases}-OH\ (C-2)\\-COOH\ (C-28)\end{cases}$, there was prepared acetyl ursolyl amide and acetyl ursolyl nitrile, both of which were reduced by lithium aluminum hydride to an ursolyl amine (C—28), $C_{29}H_{46}\begin{cases}-OH\\-CH_2NH_2\end{cases}$. By a similar reduction of ursonic acid oxime there was obtained an isomeric ursolyl amine (C—2), $C_{29}H_{46}\begin{cases}-NH_2\\-CH_2OH\end{cases}$. Methyl ursonate, $C_{29}H_{45}\begin{cases}=O\\-COOCH_3\end{cases}$, was reduced, by the Huang-Minlon modification of the Wolff-Kishner method, to ursanic acid, $C_{29}H_{45}\begin{cases}=H_2\\-COOH\end{cases}$, which was reduced by lithium aluminum hydride to the primary alcohol, ursanol, $C_{29}H_{46}\begin{cases}-H\\-CH_2OH\end{cases}$, an isomer of the naturally occurring secondary alcohol, α-amyrin, $C_{29}H_{46}\begin{cases}-OH\\-CH_3\end{cases}$.

THE COMBINATION of certain amines, such as procaine, with penicillin to form salts of low water solubility to prolong the action of penicillin in the body is well known. Since ursolic acid itself is of extremely low solubility it appeared possible that an amine obtained from it might be used in the same way. Each of the two reactive groups in ursolic acid, namely the carboxyl at C—28 and the secondary alcohol at C—2, offers points for introduction of the amino group; one of the objects of this study was to prepare these two indicated amino derivatives.

Among the known triterpene compounds in nature there has been shown to exist relationships which are of biogenetic significance. Thus, in addition to the hydroxy acid, ursolic acid, there is also found the corresponding diol, uvaol, and the secondary alcohol, α-amyrin. There has not yet been found (nor may it necessarily exist in nature) the corresponding isomeric primary alcohol; another object of this study was to prepare the indicated primary alcohol, which we have designated as *ursanol*.

Acetyl ursolyl amide was obtained in consistently good yields by the action of ammonia gas on a dioxane solution of acetyl ursolyl chloride and the presence of the amide group was confirmed by its infrared spectrum (Fig. 1). By the action of acetic anhydride acetyl ursolyl amide was converted to acetyl ursolyl nitrile, presence of the nitrile group being confirmed by its infrared spectrum (Fig. 2). Both acetyl ursolyl amide and acetyl ursolyl nitrile were reduced in good yields to an ursolyl amine (C—28) by lithium aluminum hydride.

An isomeric ursolyl amine (C—2) was prepared by reduction of ursonic acid oxime, and its methyl

* Received March 16, 1953, from the School of Pharmacy, University of Wisconsin, Madison.
Supported in part by a research grant from the National Cranberry Association, Hanson, Massachusetts.
† National Cranberry Association Fellow in Pharmaceutical Chemistry, 1951–52.

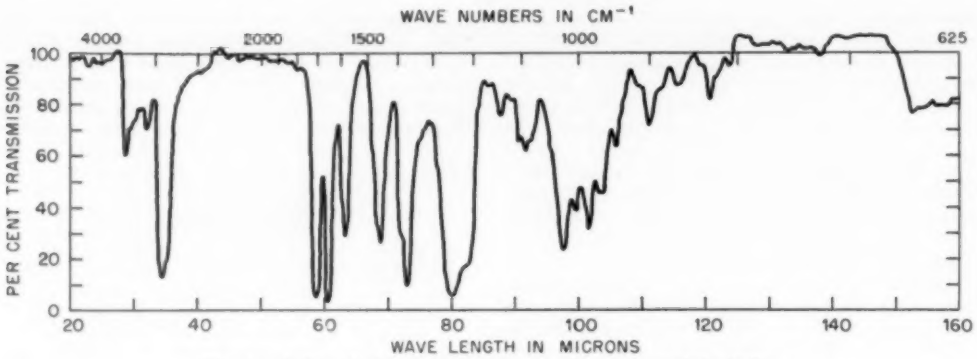


Fig. 1.—Infrared absorption spectrum of acetyl ursolyl nitrile.

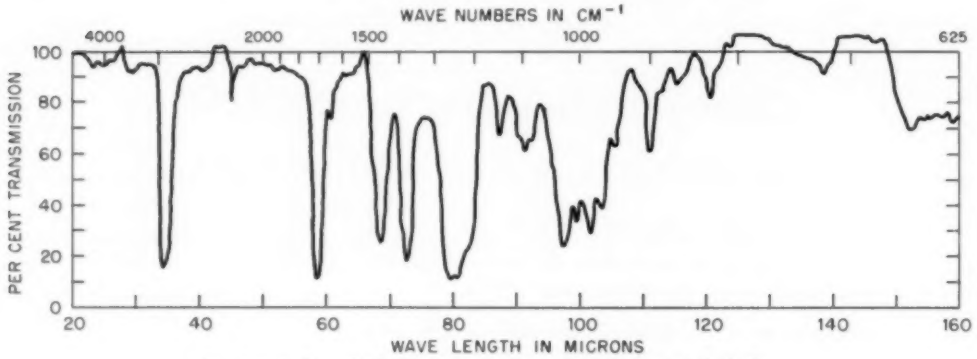


Fig. 2.—Infrared Absorption spectrum of acetyl ursolyl nitrile.

ester, by lithium aluminum hydride, the carboxyl at C—28 being reduced simultaneously to primary alcohol.

By means of the Huang-Minlon modification of the Wolff-Kishner method (1) methyl ursonate was reduced to ursanic acid which, as such and as its methyl ester, was reduced by lithium aluminum hydride to the primary alcohol, *ursanol*. The latter could not be crystallized but it yielded a crystalline *p*-nitrobenzoate.

The acetyl ursolyl amide, ursolyl amide, acetyl ursolyl nitrile, ursolyl nitrile, ursolyl amine (C—28), ursolyl amine (C—2), and ursanol prepared in this study are new compounds which have not been reported previously.

EXPERIMENTAL

Materials.—Crude ursolic acid was obtained from the National Cranberry Association and was purified by the method previously described (2).

Acetyl ursolic acid, m. p. 289–290°,[1] was prepared (3) in yields ranging from 90–96%.

Acetyl ursolyl chloride, m. p. 215-216°, was prepared (4, 5) in yields of crude product, m. p. 206-211° of 92–98%.

Methyl ursolate, m. p. 170-171°, was prepared by use of dimethyl sulfate (6) in yields of 63% and by use of diazomethane in yields of 89%.

Ursonic acid, m. p. 284-286°, was prepared (7) in yields of 68–72%; ursonic acid oxime m. p. 273-275°, prepared by action of hydroxylamine hydrochloride on ursonic acid in alcohol in presence of pyridine (8).

Methyl ursonate, m. p. 194-195°, was prepared by chromic acid oxidation of methyl ursolate (7) in yields of 68%, and by action of diazomethane on ursonic acid.

Anal.[2]—Calcd. for $C_{31}H_{48}O_3$: C, 79.42; H, 10.33. Found: C, 79.36; H, 10.38. $[\alpha]_D^{24°} = +85.0°$ (pyridine, C = 1.106); $[\alpha]_D^{25°} = +93.76°$ (chloroform, C = 0.722).

Methyl ursonate oxime, m. p. 242-244°, was prepared by action of diazomethane on ursonic acid oxime and, m. p. 244-246°, by action of hydroxylamine hydrochloride on methyl ursonate (7).

Acetyl Ursolyl Amide.—One gram of crude acetyl ursolyl chloride was dissolved in 50 ml. of dry dioxane and ammonia gas was passed into the solution for approximately one hour. The light brownish-white precipitate which formed was removed by filtration, the filtrate decolorized with Norit and concentrated to a small volume, from which there separated a crystalline mass. Two recrystallizations from absolute methanol yielded 0.7 Gm. (73%) of large hexagonal crystals which, by repeated re-

[1] All melting points were obtained with Anschutz total immersion thermometers.

[2] We are indebted to Professor A. L. Wilds, Chemistry Department, University of Wisconsin, for combustion data.

crystallization from the same solvent gave colorless prismatic crystals, m. p. 293.5–294.5°.

Anal.—Calcd. for $C_{32}H_{51}O_2N$: C, 77.26; H, 10.26. Found: C, 77.12; H, 10.14. $[\alpha]_D^{21°} = +45.2°$ (chloroform, C = 1.184). The characteristic dual nature of the stretching at 3μ in the infrared spectrum (in chloroform, Fig. 1)[1] does not exclude the presence of an N-substituted amide (9); however the method of preparation of acetyl ursolyl amide eliminates that possibility.

Ursolyl Amide.—One gram of acetyl ursolyl amide was refluxed with 50 ml. of 10% methanolic potassium hydroxide for forty hours, the mixture evaporated to dryness, and the crystalline residue washed repeatedly with hot water. The remaining residue was dissolved in 100 ml. of ether, the solution shaken out with dilute aqueous sodium hydroxide to remove any free ursolic acid, dried, and evaporated. Crystallization of the resulting residue from absolute methanol and from alcohol-acetone-water yielded hexagonal scale crystals, m. p. 281.5–283.5°, $[\alpha]_D^{23°} = +61.3°$ (chloroform, C = 1.174). Acetylation of this compound with acetic anhydride in presence of pyridine at room temperature in the usual manner yielded the same acetyl ursolyl amide described above.

Attempts to hydrolyze the amide group of both acetyl ursolyl amide and ursolyl amide to obtain ursolic acid, using both acid and alkaline agents, were unsuccessful, indicating that the amide group of these compounds is very resistant to hydrolysis.

Acetyl Ursolyl Nitrile.—A mixture of 1.5 Gm. of acetyl ursolyl amide, 1.5 Gm. of anhydrous sodium acetate and 15 ml. of acetic anhydride was refluxed for four hours, then poured into 250 ml. of water and cooled in the refrigerator for several hours. The crystalline solid which formed was crystallized from absolute methanol to yield 1.06 Gm. (74%) of white silky needles, m. p. 247–248°.

Anal.—Calcd. for $C_{32}H_{49}O_2N$: N, 2.92. Found (Kjeldahl): N, 2.84; 2.98. $[\alpha]_D^{21°} = +76.9°$ (chloroform, C = 1.378). The characteristic stretching at 4.5μ in the infrared spectrum (in chloroform, Fig. 2) shows the presence of the nitrile group in the compound (9).

In one attempt to prepare acetyl ursolyl amide by the action of liquid ammonia on acetyl ursolyl chloride there was obtained, instead, in 51% yield a product which was shown by m. p., optical rotation, carbon, and hydrogen values and infrared spectrum, to be acetyl ursolyl nitrile. Although this compound could not be obtained again under the same conditions, its formation may be explained in this one case according to Whitmore (10).

Ursolyl Nitrile.—A mixture of 0.3 Gm. of acetyl ursolyl nitrile and 30 ml. of 10% methanolic potassium hydroxide was refluxed for twenty-three hours, the mixture evaporated to dryness and the white residue washed repeatedly with water. The remaining solid was dissolved in 50 ml. of ether, the solution shaken out with dilute aqueous sodium hydroxide, dried, and evaporated. Repeated recrystallization from absolute methanol yielded hexagonal crystals, m.p. 260.5–261.5° which, after drying for eighteen hours at 100°/0.5 mm., melted at 255.5–256.5°.

[1] We are indebted to Dr. E. E. van Tamelen, Chemistry Department, University of Wisconsin, for infrared data.

Anal.—Calcd. for $C_{30}H_{47}ON$: C, 82.38; H, 10.76. Found: C, 82.39; H, 10.64. $[\alpha]_D^{23°} = +86.4°$ (chloroform, C = 2.546).

Ursolyl Amine (C—28).—To a solution of 2.5 Gm. (excess) of lithium aluminum hydride in 700 ml. of anhydrous ether in a 1,000-ml. three-necked flask equipped for this purpose (11), was added a solution of 1.5 Gm. of acetyl ursolyl amide in 60 ml. of tetrahydrofuran (12) at a rate sufficient to produce gentle reflux; the mixture was then refluxed with stirring for an additional twenty-five hours, the excess hydride decomposed by addition of 50 ml. of water, and the metal complex hydrolyzed by addition of 100 ml. of 5% aqueous sodium hydroxide solution. The ether layer was separated, the alkali layer washed repeatedly with ether, the combined ether solutions dried and evaporated to yield 1.22 Gm. (92%) of white crystalline residue which, by repeated crystallization from absolute methanol, was obtained as white scale-like crystals, m. p. 201–201.5°.

Reduction of acetyl ursolyl nitrile in a similar manner gave the same product in 86% yield.

Anal.—Calcd. for $C_{30}H_{51}ON$: C, 81.56; H, 11.63; N, 3.17. Found: C, 81.22; H, 11.45; N (by perchloric acid titration) (13), 3.05. $[\alpha]_D^{23°} = +69.7°$ (chloroform, C = 3.156).

The amine hydrochloride, prepared by action of hydrogen chloride gas on an ether solution of ursolyl amine (C—28), was obtained as a white amorphous product, with no sharp melting point.

Anal.—Calcd. for $C_{30}H_{51}ON.HCl$: N, 2.93. Found: N, 2.83 (by amperometric titration with silver nitrate solution).

Ursolyl Amine (C—2).—Initial attempts to reduce ursonic acid oxime by lithium aluminum hydride (14), in the manner described for the reduction of acetyl ursolyl amide above, resulted in a mixture of crude amine and unreduced oxime which was difficult to separate. In a modification of the above procedure 1.2 Gm. of ursonic acid oxime was reduced in the same manner, using a reflux time of twenty-five hours and, after decomposing the excess hydride by addition of water, the metal complex was decomposed by the addition of 100 ml. of dilute sulfuric acid, which also formed the amine sulfate which was insoluble in both the aqueous and ether layers. This salt was collected by filtration, suspended in 200 ml. of ether, and the suspension shaken with two successive 20-ml. portions of 10% aqueous sodium hydroxide solution. The ether layer, containing the free amine, was washed with water, dried, and evaporated, leaving a resin-like residue. This crude product was purified by conversion to the sulfate and regeneration to the free amine which, by crystallization from diluted methanol yielded 0.78 Gm. (71%) of pure ursolyl amine (C—2) as white flakes, m. p. 153.5–154.5°. From the original ether layer remaining after the reduction there was recovered 0.19 Gm. (16% of the starting quantity) of unreacted ursonic acid oxime.

Reduction of 1.26 Gm. of methyl ursonate oxime in a similar manner, using a reflux time of twenty hours, yielded 0.88 Gm. (77%) of the same product.

Anal.—Calcd. for $C_{30}H_{51}ON$: C, 81.56, H, 11.63. Found: C, 81.08; H, 11.40. $[\alpha]_D^{24°} = +70.06°$ (chloroform, C = 0.334).

The amine hydrochloride, prepared by action of hydrogen chloride gas on an ether solution of ur-

solyl amine (C—2), was crystallized from methanol-ether (1:3) as white needles which decomposed without melting below 355°.

Anal.—Calcd. for $C_{30}H_{51}ON.HCl$: C, 75.33; H, 10.96; N, 2.93. Found: C, 75.37; H, 10.83; N, 2.93 (by perchloric acid titration).

Ursanic Acid.—After several unsuccessful attempts at the Clemmensen reduction of methyl ursonate, under various conditions (15), ursanic acid was obtained by the Huang-Minlon modification of the Wolff-Kishner procedure (1) as follows: To a solution of 1.8 Gm. of methyl ursonate in 100 ml. of absolute ethanol was added 140 ml. of diethylene glycol and 6 ml. of 85% hydrazine hydrate, the mixture was refluxed for fifteen minutes, then 14 Gm. of solid sodium hydroxide was added and the mixture heated for one hour on the steam bath. The flask was then transferred to a heated sand bath, the condenser removed, heated until the temperature of the mixture rose to 190–200°, the condenser connected again, and the mixture refluxed again for five to six hours. To the white gel-like substance which formed in the flask upon cooling there was added 200 ml. of absolute methanol, the contents heated to obtain a clear solution, acidified with dilute hydrochloric acid, and diluted with one liter of water which caused the product to separate as a white precipitate. By filtration there was obtained 1.68 Gm. (99%) of product, m. p. 227–229°. Although ursanic acid has been reported (7) as needle crystals, m. p. 223–225°, all attempts to crystallize our product were unsuccessful, even after purification through formation of the crystalline ammonium, sodium, or potassium salts. $[\alpha]_D^{23°}$ = +67.4° (chloroform, C = 1.052).

Methyl Ursanate.—To an ether solution of excess diazomethane was added 0.4 Gm. of ursanic acid in 200 ml. of ether, the solution allowed to stand at room temperature for twenty-four hours, washed with successive portions of 5% aqueous sodium hydroxide solution and water, dried, and evaporated. The residue was crystallized from isopropyl alcohol or from absolute methanol to yield 0.37 Gm. (90%) of methyl ursanate as white needles and from ethanol acetone (3:1) as prisms, m. p. 122–123°. Purity and homogeneity of the product were established by chromatography on alumina.

Anal.—Calcd. for $C_{31}H_{50}O_2$: C, 81.87; H, 11.09. Found: C, 81.88; H, 11.06. $[\alpha]_D^{22°}$ = +80.8° (pyridine, C = 0.866). Jacobs and Fleck (7) reported for their product, m. p. 117–118°, $[\alpha]_D^{23°}$ = +65° (pyridine, C = 1.020).

Ursanol.—Reduction of 1.3 Gm. of ursanic acid, dissolved in 120 ml. of ether, was carried out in the manner described for the reduction of acetyl ursolyl amide above, using a solution of 2 Gm. of lithium aluminum hydride in 700 ml. of anhydrous ether, a reflux time of twenty hours, and decomposing the metal complex by addition of 100 ml. of dilute sulfuric acid. The ether layer was separated, the acid layer washed repeatedly with ether and the combined ether solutions were shaken with several successive portions of 5% aqueous sodium hydroxide solution which caused the separation of a considerable amount of white precipitate; this was shown to be the sodium salt of unreacted ursanic acid. Evaporation of the washed ether solution yielded an oily residue which was dried in vacuum to 0.6 Gm. (48%, calcd. as $C_{30}H_{50}O$) of amorphous solid. Attempts to crystallize this product from a variety of solvents were unsuccessful.

In another experiment the reduction of 1.1 Gm. of methyl ursanate, dissolved in 50 ml. of tetrahydrofuran, was carried out with lithium aluminum hydride in a similar manner using a reflux time of thirty-two hours. The ether layer from this reduction after washing, drying, and evaporating yielded 0.97 Gm. (94%) of amorphous product, m. p. 83–84°, which resisted all efforts at crystallization. Chromatography of the product on alumina, using 2.5% ethanol in benzene for elution, established its purity and homogeneity.

Anal.—Calcd. for $C_{30}H_{50}O$: C, 84.51; H, 11.73. Found: C, 84.21; H, 11.71. $[\alpha]_D^{23°}$ = + 73.3° (chloroform, C = 0.996).

The *p*-nitrobenzoate of ursanol prepared by reaction with *p*-nitrobenzoyl chloride in pyridine solution, crystallized from Skelly-B as small yellowish-white needles, m. p. 101–102°.

REFERENCES

(1) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487(1946); **71**, 3301(1949).
(2) Wu, Betty Y. T., and Parks, Lloyd M., THIS JOURNAL, **42**, 602(1953).
(3) Sando, C. E., *J. Biol. Chem.*, **90**, 477(1931).
(4) Goodson, J. A., *J. Chem. Soc.*, 999(1938).
(5) Sell, H. M., and Kremers, R. E., *J. Biol. Chem.*, **125**, 451(1938).
(6) Orr, J. E., *et al.*, THIS JOURNAL, **34**, 39(1945).
(7) Jacobs, W. A., and Fleck, E. E., *J. Biol. Chem.*, **92**, 487(1931).
(8) Kuwada, S., and Matsukawa, T., *J. Pharm. Soc. Japan*, **53**, 596(1933).
(9) Colthup, N. B., *J. Opt. Soc. Amer.*, **40**, 397(1950).
(10) Whitmore, F. C., "Organic Chemistry," 2nd ed., Van Nostrand, New York, 1951, p. 417.
(11) Usser, A., and Schlettler, E., *Helv. chim. acta.*, **31**, 1397(1948).
(12) Wu, Betty Y. T., and Parks, Lloyd M., THIS JOURNAL, **39**, 475(1950).
(13) Higuchi, T., and Concha, J., *Science*, **113**, 210(1951); THIS JOURNAL, **40**, 173(1951).
(14) Larsson, E., *Svensk. Kem. Tidskr.*, **61**, 242(1949).
(15) Adams, R., "Organic Reactions," Vol. I, John Wiley & Sons, Inc., New York, 1942, p. 155.

Observations on the Antispasmodic Principle of *Euphorbia pilulifera*, L.*

By FLOYD P. HALLETT† and LLOYD M. PARKS

In attempts to isolate the antispasmodic and "spike" principles of *Euphorbia pilulifera* it has been found that the former is stable to acids but destroyed by alkali, is dialyzable, is not adsorbed by cation exchange resins, and can be extracted from aqueous solutions by prolonged extraction with amyl alcohol. The spike principle is unstable on standing in aqueous solution, is dialyzable, and is adsorbed by cation exchange resins, from which it can be eluted by 5 per cent HCl.

RECENTLY Hazleton and Hellerman (1, 2) have re-evaluated the pharmacology of *Euphorbia pilulifera*, L., using improved modern techniques. Their experiments on dealcoholized fluidextracts of Euphorbia showed the presence of two physiologically active principles which were characterized by their actions on isolated histamine contracted guinea-pig ileum. The first principle caused a transitory additional spike-like contraction while the second caused complete relaxation of the histamine contraction. These principles were designated as the "spike" principle and the antispasmodic principle. They found that the spike principle was adsorbed by Lloyd's Reagent while the antispasmodic principle was not, that the antispasmodic principle was not extracted from aqueous solutions by ether at *p*H 4.5, was not destroyed by boiling with dilute hydrochloric acid, and was stable over long periods of time at room temperature in acidic aqueous solution.

Previous chemical investigations of *Euphorbia pilulifera* have not resulted in the isolation of any pure substance which might be responsible for these physiological actions of the drug. The work reported here was undertaken with the aim of learning more about the chemical properties of the active principles and in an attempt to isolate the antispasmodic principle.

EXPERIMENTAL

Testing for Antispasmodic Activity.—Antispasmodic activity in the various fractions obtained in the isolation work was followed by noting the relaxation of isolated histamine contracted guinea-pig ileum essentially in the same manner as that described by Hazleton and Hellerman (1). After standardization of the histamine[1] dose (0.25 to 3.0 μg. of histamine base) a quantity of fluidextract or isolated fraction representing 1 to 2 Gm. of crude drug was added to the muscle chamber. Presence of antispasmodic activity was concluded when a sample caused relaxation of the histamine contraction to the base line in successive tests. All fractions were tested in aqueous solution after removal of alcohol or other solvent by vacuum distillation and taking up the residue in water.

Extractions of the Crude Air-Dried Drug

Percolation with Dilute Alcohol.—A fluidextract of the drug (1), from which the alcohol was removed by vacuum distillation and the water-insoluble resin removed by centrifuging, resulted in a dark brown solution (*p*H 4.5) which produced the characteristic spike and relaxant actions on histamine contracted muscle.

Continuous Extraction with Alcohol.—The drug was extracted for thirty hours with ethyl alcohol in a Soxhlet apparatus. The alcohol was removed from the extract in the usual manner and the aqueous solution of the residue produced a large additional contraction on muscle which was already contracted by histamine. After filtration through Lloyd's Reagent this solution produced only relaxation of the histamine contracted muscle similar to that obtained with the diluted alcohol percolate after the same treatment. Further extractions of the marc by percolation with diluted alcohol and by Soxhlet extraction with alcohol indicated that the marc contained slight additional amounts of the spike principle but no antispasmodic principle. Since the extract obtained by Soxhlet extraction contained the desired antispasmodic principle with less extraneous materials it was used in the subsequent experimental work which is reported below and will be designated as the alcohol-free extract (*A*).

Other Methods and Solvents.—Maceration of the drug by refluxing with successive portions of alcohol was shown to extract both active principles. Maceration with hot water yielded both principles but the extract contained large amounts of inactive mucilaginous materials. Soxhlet extractions with Skelly B and with ether removed neither principle from the drug. Results of macerations with methyl alcohol, acidified methyl alcohol, and acetic acid were inconclusive due to interference with the muscle test by traces of these solvents.

Tests and Observations on the Alcohol-free Extract (*A*)

Stability of the Antispasmodic Principle.—When (*A*) was allowed to stand at room temperature for several months there had formed in it considerable mold growth. When filtered from this growth the

* Received March 16, 1953, from the School of Pharmacy, University of Wisconsin, Madison.
Supported in part by a research grant from Templetons Ltd., Toronto, Canada, and Templetons, Inc., Buffalo, N. Y.
† American Foundation for Pharmaceutical Education Fellow. Present address: Mallinckrodt Chemical Works, St. Louis, Mo.
[1] Prepared by diluting the contents of ampuls of histamine phosphate, U. S. P. XIV (Eli Lilly & Co.).

solution showed no loss of antispasmodic activity. Antispasmodic activity remained unchanged in a portion of (*A*) which was allowed to stand over chloroform for two years. It was also stable when (*A*) was heated for long periods of time with varying amounts of hydrochloric acid. Oxygen, when bubbled through (*A*) for several hours, produced no loss of activity. However, when (*A*) was distilled *in vacuo* in the presence of Lloyd's Reagent and the residue thus obtained was powdered and dried at 80°, aqueous extracts of the thus dried powdered material possessed no antispasmodic activity.

A portion of (*A*) (*p*H 4.15), after filtration through Lloyd's Reagent, was adjusted to *p*H 7.2 with saturated $NaHCO_3$ solution and the yellow-green precipitate which formed was removed by filtration. The filtrate showed no relaxant activity either before or after adjustment of its *p*H back to 4.15; treatment of the precipitate with acidified water partially dissolved it but the acid solution was also devoid of activity. Inactivation of the antispasmodic principle by the slight alkalinity of Locke-Ringer Solution was also demonstrated. The addition of 2 ml. of (*A*) to 200 ml. of Locke-Ringer Solution caused the *p*H to drop from 7.8 to 7.1. When 2 ml. of (*A*) was added to the muscle chamber immediately prior to the addition of a standardized dose of histamine, protection of the muscle was evidenced by the appearance of only a very small histamine contraction. However, when 2 ml. of (*A*) was allowed to stand with 200 ml. of Locke-Ringer Solution in the muscle chamber for two hours prior to the addition of the same dose of histamine, it furnished no protection of the muscle.

Dialysis.—Dialysis of (*A*) was carried out in a large cylindrical graduate. The solution outside the dialyzing bag[3] was removed and replaced with fresh water at eight-hour intervals. The combined solutions from three eight-hour dialysis runs, upon concentration, were found to contain nearly all of the antispasmodic activity originally present in (*A*). Much of the color and extraneous material remained in the solution inside the bag.

Tests with Ion-exchange Resins.—The possibility of the antispasmodic principle being basic in nature was suggested by the basic nitrogen nature of the well-known antispasmodic and antihistaminic compounds. For this reason adsorption of the principle on cationic exchange resins was investigated. Amberlite IR-120 in a chromatographic column was converted to the hydrogen form with 5% HCl and washed free of the acid with double distilled water. When (*A*) was passed through this column the spike principle was adsorbed but the antispasmodic principle appeared in the effluent. Elution of the spike principle was achieved by use of 5% HCl, the adsorbed spike principle from 50 ml. of (*A*) appearing in the first 50 ml. of eluate. The eluted material, obtained by evaporation of the solvent and removal of last traces of HCl by evaporating with successive portions of absolute alcohol, consisted of a mixture of crystalline and amorphous material, the chief constituent of which was potassium chloride. Approximately one-half of this mixture dissolved when it was treated with hot absolute alcohol and muscle tests showed that the spike principle had gone into the alcoholic solution.

[3] "Visking" Cellulose Sausage Casing.

Amberlite IRC-50 also passed the antispasmodic principle but adosrbed the spike principle from (*A*) less efficiently, however, than did IR-120. Basic resins, Amberlite IRA-400 and Ionac A-300, destroyed the antispasmodic acitivity presumably due to their alkalinity.

The antispasmodic principle was not adsorbed from (*A*) by aluminum oxide in a chromatographic column.

Attempted Clarification with Lead Salts.—Treatment of (*A*) with lead acetate solution in the conventional manner, removal of the precipitate by centrifuging, passage of the resulting pale yellow supernatant solution through an IR-120 resin column to remove excess lead, and adjustment of the eluate to *p*H 4.15 by addition of 5% $NaHCO_3$ solution, yielded a solution which produced a reversible "deadening" of histamine contracted muscle, characterized by immediate relaxation, loss of normal rhythmic activity, and lack of response to relatively large subsequent doses of histamine. Blank tests showed that this deadening effect was due partly to the presence of free acetic acid resulting from removal of lead ion in the resin column. No means was found to remove this acetic acid without, at the same time, destroying the relaxant principle.

The pale yellow solution, which resulted from clarification of (*A*) with freshly prepared lead hydroxide suspension (from ammonium hydroxide and lead nitrate) and removal of excess lead by the IR-120 resin, produced the same deadening effect on muscle. The fact that blank tests with lead hydroxide and lead carbonate produced the same effect, indicated that traces of lead, not removed by the resin column, also interfered with the muscle test.

Extraction with Organic Solvents.—The repeated shaking of portions of (*A*) in a separatory funnel with various organic solvents failed to cause partition of the antispasmodic principle into ether, chloroform, chloroform and alcohol mixtures, ethyl acetate, butanol, or amyl alcohol. These same solvents were used for the prolonged extraction of concentrated portions of (*A*), (1 ml. equivalent to 3 Gm. of crude drug), using a continuous liquid-liquid extractor. Only butanol and amyl alcohol extracted determinable amounts of antispasmodic activity from (*A*) in this manner. Butanol also extracted considerable water, coloring matter, and extraneous materials. Amyl alcohol extraction was allowed to proceed for eighteen hours. Addition of ether to the amyl alcohol extract caused the precipitation of inactive syrupy material. The ether was removed from the remaining liquid by distillation and the amyl alcohol was removed by steam distillation. During the latter procedure considerable black resinous material was precipitated. Centrifuging of the residue resulting from the steam distillation yielded a clear orange-yellow colored aqueous supernatant solution which completely relaxed histamine contracted muscle and appeared to contain most of the antispasmodic activity of the original portion of (*A*). A fifty-hour extraction with amyl alcohol appeared to remove a larger portion of the relaxing principle but the final solution from this extraction was contaminated with a much greater amount of extraneous material.

Properties of the Spike Principle.—Various properties of the spike principle were observed during

attempts to isolate the antispasmodic principle. The spike principle appeared to be extracted from the crude drug in larger amounts by alcohol than by diluted alcohol or water. It was stable to heat and to acids and alkalies for short periods of time. Aqueous acidic solutions containing the principle showed loss of the characteristic spike activity after standing for several weeks but in hydroalcoholic solution or in aqueous solutions stored over chloroform there was no loss of spike activity after standing for several months. The spike principle easily passed through a dialyzing membrane, was adsorbed by Lloyd's Reagent, from which attempts to elute it were unsuccessful, and was adsorbed on cation exchange resins from which it could be eluted with 5% HCl. Other properties indicated that it might be a very weakly basic substance.

DISCUSSION

Sensitivity of the antispasmodic principle to alkaline conditions eliminated a large number of possible isolation procedures and its great solubility in water and insolubility in nonpolar solvents makes it difficult to effect its partition into an organic solvent.

That the antispasmodic activity is associated with a strongly basic substance seems unlikely, because of its behavior toward cation exchange resin. This does not eliminate the possibility of an alkaloid, since a weakly basic compound might be present or the acid *p*H of the solution as it passes through the resin column might prevent adsorption of some alkaloids. A glycosidal type is unlikely, because of retention of activity when the principle is heated with acid.

Passage through IR-120 resin of solutions which showed no spike activity appeared to enhance the relaxant activity. This may have been due to either removal of minimal amounts of the spike principle or its decomposition products, some chemical change produced by exposure of the substance to very acidic conditions, or a change in physiological activity due to removal of cations.

SUMMARY

1. Isolation procedures have been applied to *Euphorbia pilulifera*, L.

2. Properties of the physiologically active principles of the plant are described.

REFERENCES

(1) Hazleton, L. W., and Hellerman, R. C., THIS JOURNAL **37**, 491(1948).

(2) Hazleton, L. W., and Hellerman, R. C., *ibid.*, **39**, 147 (1950).

Trihexyphenidyl—Experimental Studies Employing a Muscle-Nerve Preparation*

By O. D. PRIDDLE, Jr.† and Y. T. OESTER

Evidence is presented to indicate that trihexyphenidyl, when injected intravenously in dogs, produces a decrease in muscle response to applied electrical stimuli. It has been demonstrated that this decrease in muscle response is due to activity of trihexyphenidyl above the nerve-spinal cord-muscle reflex arc.

DOSHAY (1) outlined the need for more effective therapeutic agents in the treatment of Parkinsonism; Cunningham (2) described a new antispasmodic which was 3-(1-piperidyl)-1-cyclohexyl-1-phenyl-1-propanol hydrochloride. It has been called trihexyphenidyl and been given the trade name of Artane.

Reports on the clinical use of trihexyphenidyl (3, 4) indicate that it may be of value in the treatment of Parkinsonism, spasmodic torticollis, facial spasm and miscellaneous dyskinesias. The present research work was done in the endeavor to extend the present pharmacological knowledge of this new agent which has been recommended as an antispasmodic.

METHODS AND PROCEDURES

Adult dogs were used in this study. The dogs were anesthetized with 70–85 mg./Kg. of Dial by intraperitoneal injection. Blood pressure recordings were made from the common carotid artery by the use of an arterial cannula attached to a mercury manometer through an 8.0% sodium citrate anticoagulant solution.

The femoral vein was cannulated and attached to a graduated burette. All injections were made through this venous cannula. The indicated drug was added to the burette and then flushed into the circulation with 20 cc. of nonpyrogenic, sterile, normal saline.[1]

* Received March 11, 1953, from the Department of Pharmacology, Stritch School of Medicine, Loyola University, Chicago, Illinois.

† Present address: Mellon Institute, Pittsburgh, 13, Pa.

[1] Sterile, nonpyrogenic, normal saline was supplied through the courtesy of the Baxter Laboratories, Chicago, Illinois.

The arrangements for recording the contractions of the anterior tibialis muscle were as follows: the posterior tibial nerve and the common peroneal nerve were exposed in the thigh; the posterior tibial nerve was sectioned in the region of the knee; shielded electrodes from the stimulator (Golseth-Fizzell Constant Impulse Stimulator) were affixed to the proximal cut end of the tibial nerve; the tendon of the anterior tibialis muscle was exposed and sectioned near its attachment. The cut tendon was attached to the recording lever by a metal hook, and the recording lever pulled against an elastic spring. The leg was securely held by means of two screws placed in the tibia and femur, respectively, which were fixed to a support firmly attached to the table. Stimuli were applied either to the posterior tibial nerve, and therefore anatomically would pass through a nerve-spinal cord-muscle arc (hereafter referred to as a reflex stimulation), or to the common peroneal nerve and therefore an atomically would pass through a nerve-muscle unit (hereafter referred to as an indirect stimulation). Either stimulus resulted in a contraction of the anterior tibialis muscle.

The electrical stimuli required to produce an adequate response varied from 0.5–5.0 milliamperes of direct current. The strength of the stimuli was kept constant during any one experiment, but was at time maximal in strength. The stimuli were applied automatically at the rate of one impulse every second. This results in an effective time basis for the records obtained. A 0.4% suspension of trihexyphenidyl[1] was found to have a *p*H of 6.38. Gentle heating proved to be a satisfactory method for putting the 0.4% suspension into the form of a stable solution at room temperature.

RESULTS

The data obtained from the studies on sixteen dogs may be briefly summarized in the following manner. With all doses of trihexyphenidyl used (1–20 mg./Kg.) a decrease in salivation was grossly observed. With intravenous doses of trihexypenidyl in the range of 1–5 mg./Kg., a drop in blood pressure was routinely observed. The magnitude of the fall in blood pressure varied from 10 mm. to 30 mm. of mercury in twenty-one observations (Fig. 1). When 10-15 mg./Kg. doses of trihexyphenidyl were administered intravenously, the fall in blood pressure increased proportionately to the dosage used in twelve observations. Near fatal doses of trihexyphenidyl (20–25 mg./Kg.) produced a pronounced and persistent fall in blood pressure of the order of 20 to 75 mm. of mercury in eighteen observations (Figs. 2 and 3).

When the anterior tibialis muscle was stimulated, either by the reflex route (posterior tibial nerve), or by the indirect route (common peroneal nerve), there was a consistent slight transitory increase in the magnitude of the response with doses of trihexyphenidyl in the range of 1–5 mg./Kg. when administered intravenously.

Following larger intravenous doses of trihexyphenidyl (10–20 mg./Kg.) a decrease in the magnitude of the response of the anterior tibialis muscle

[1] Trihexyphenidyl was supplied through the courtesy of the Lederle Laboratories, Pearl River, New York.

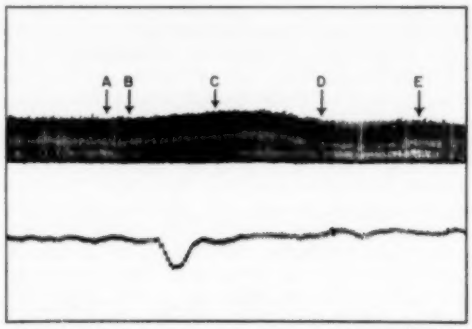


Fig. 1.—Effect of 1 mg./Kg. of trihexyphenidyl intravenously on the reflex anterior tibialis muscle preparation, 10.2 Kg., male, dog, Dial anesthesia. Upper tracing—muscle response. Lower tracing—blood pressure. *A* to *B*—1 mg./Kg. of trihexyphenidyl intravenously. *C-D-E* intervals of one, two, and three minutes after injection.

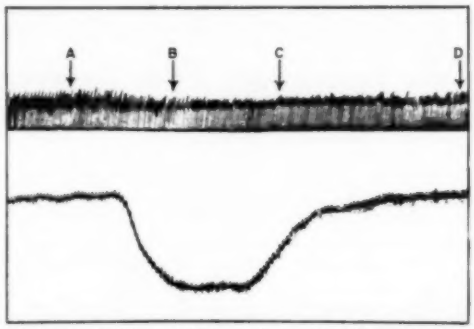


Fig. 2.—Effect of 20 mg./Kg. of trihexyphenidyl intravenously on the indirect anterior tibialis muscle preparation. 12.0 Kg., female, dog. Dial anesthesia. Upper tracing—muscle response. Lower tracing—blood pressure. *A* to *B*—20 mg./Kg. of trihexyphenidyl intravenously. *C*—one minute injection. *D*—three minutes after injection of trihexyphenidyl.

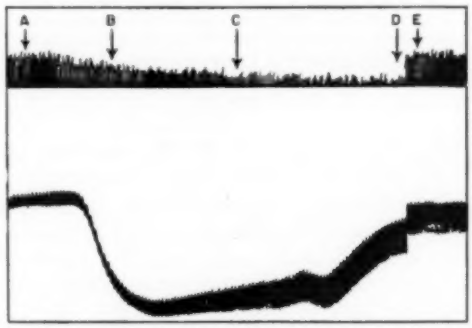


Fig. 3.—Effect of 20 mg./Kg. of trihexyphenidyl intravenously on the reflex anterior tibialis muscle preparation, 9.0 Kg., male, dog. Dial anesthesia. Upper tracing—muscle response. Lower tracing—blood pressure. *A* to *B*—20 mg./Kg. of trihexyphenidyl intravenously. *C*—one minute after injection. *D*—record interrupted for twelve minutes. *E*—return to preinjection level twelve minutes after injection.

occurred during either reflex or indirect stimulation. This decrease was pronounced when the reflex stimulation was employed (Fig. 3). However, in contrast, when indirect stimulation was employed, a decrease in the magnitude of the response of the anterior tibialis muscle was noted which was markedly smaller than that produced with the reflex stimulation. If the dog survived the injection, the muscle response returned to the preinjection level in twenty to thirty minutes. In an endeavor to demonstrate what effect if any, the blood pressure lowering itself may produce on the reflex stimulation, the following experiment was performed. A fall in blood pressure was produced with sodium nitrite which corresponded to that noted with 20 mg./Kg. of trihexyphenidyl. There was no change in the amplitude of either the reflex of the indirect muscle-nerve response. As a means of checking the reflex circuit being used, the sciatic nerve was severed, at the termination of the experiment. There was an immediate failure in the reflex response. This demonstrated that the previously used reflex preparation was functional. Stimulation of the common peroneal nerve on the same animal, produced excellent responses. This indicates that the indirect circuit was intact and functional.

DISCUSSION

The general observations of interest were: (*a*) the consistent temporary fall in blood pressure; (*b*) the slight transitory increase in the muscle response following small (1–5 mg./Kg.) doses of trihexyphenidyl; (*c*) the pronounced temporary decrease in the muscle response to reflex stimulation following injections ranging from 10–20 mg./Kg. of trihexyphenidyl. The depression following indirect stimulation with the same dosages was of a minor degree.

These reactions are apparently an expression of two types of response to trihexyphenidyl. The striking depression of the muscle response which occurred following trihexyphenidyl when reflex stimulation was employed, indicates an effect on this reflex arc (Fig. 3). The much smaller depression which occurred with indirect stimulation (Fig. 2) indicates that the chief site of this depressing action is predominantly on the reflex arc, at a site other than the peripheral muscle-nerve unit.

From the experimental work presented here, one may postulate a central effect of trihexyphenidyl as well as a minor peripheral effect. It is worthy to note that the oral dosage used in human Parkinsonism (3, 4) is within the range of 0.1 mg./day. The dosages which were used in this experimental work were therefore 10 to 200 times greater than the orally administered human dose. Obviously the work reported herein has not provided any data to explain the clinical results which have been reported with the use of trihexyphenidyl in Parkinsonism except as the clinical results pertain to an effect on the central nervous system.

In the dog, no effects were seen in either the reflex or the indirect preparations, which would support the findings that trihexyphenidyl, in the small doses used in human Parkinsonism, relieves the spasms and tremors associated with the condition. If this effect is present in humans, the experimental work reported here indicates that the site of this action of trihexyphenidyl is above the reflex arc as previously defined.

SUMMARY

Studies were made of the blood pressure, and of the reflex and indirect stimulations of the anterior tibialis muscle with dogs under Dial anesthesia following the intravenous administration of trihexyphenidyl in dosages of 1–20 mg./Kg. Following the administration of the drug there was a routine fall in blood pressure which was of a transitory nature and roughly parallel to the amount of the drug administered. With doses of 1–5 mg./Kg. a slight transitory increase in the response of the anterior tibialis muscle to either reflex or indirect stimulation was noted. Larger doses, 10–20 mg./Kg., produced a decrease in muscle response which was strikingly more pronounced with the reflex preparation than with the indirect preparation, indicating activity above the reflex arc.

REFERENCES

(1) Doshay, L. J., Zigarelli, J., and Loewy, P., *Med. Record*, **1**, June(1947).
(2) Cunningham, R. W., Harned, B. K., Clark, M. C., Cosgrove, R. R., Daughtery, N. S., Hine, C. H., Vessey, R. E., and Yuda, N. N., *J. Pharmacol. Exptl. Therap.*, **95**, 151(1949).
(3) Doshay, L. J., and Constable, K., *J. Am. Med. Assoc.*, **140**, 1317(1949).
(4) Corbin, K. B., *ibid.*, **141**, 377(1949).

The Antitubercular Activity of D-Galacturonic Acid Isonicotinyl Hydrazone*

By PETER P. T. SAH and S. ANDERSON PEOPLES†

D-Galacturonic acid isonicotinyl hydrazone, a new compound, is prepared with a high yield by combining D-galacturonic acid with isonicotinic acid hydrazide. The new drug is highly active, both *in vitro* and *in vivo*, against *Mycobacterium tuberculosis* H37Rv and comparatively much less toxic than isonicotinic acid hydrazide.

IN A PREVIOUS paper, we reported that D-glucuronolactone, an agent recently recommended for the treatment of arthritis (1) and for the detoxication of a number of toxic drugs (2), may be combined with isonicotinic acid hydrazide to form a new derivative, namely, D-glucuronolactone isonicotinyl hydrazone (3), which is highly active both *in vitro* and *in vivo* against *Mycobacterium tuberculosis* H37Rv and comparatively much less toxic (4) than the parent drug, the uncombined isonicotinic acid hydrazide (5, 6).

The antitubercular activity of D-glucose isonicotinyl hydrazone was reported by two groups of investigators (7, 8). It was found to be much less toxic but also less effective than the free hydrazide. Most recently a number of isonicotinyl hydrazones derived from various sugars (9, 10) were also studied. According to these investigators, none of these derivatives were found to be as good as the free hydrazide. In our studies on the use of isonicotinic acid hydrazide as a reagent for the identification of aldehydes and ketones, we found the sugar derivatives to be much less easily prepared. In aqueous or dilute alcoholic solutions, they do not crystallize as quickly as the other isonicotinyl hydrazones, especially the aldehyde derivatives. When the aqueous solutions are concentrated, the sugar derivatives char very easily even at a temperature below that of the steam bath. Also the yields in many cases are very low.

Since D-galacturonic acid is closely related in chemical structure to D-glucuronolactone and may be prepared from waste products such as the Chinese pomelo (11) or other citrus fruit peels, it becomes a point of interest if D-galacturonic acid would also combine with isonicotinic acid hydrazide to form a hydrazone which might be a new chemotherapeutic agent for tuberculosis, even better than either isonicotinic acid hydrazide or D-glucuronolactone isonicotinyl hydrazone, which has already been undergoing clinical experiments on human patients with good, promising results.

This paper deals with (*a*) the method of preparation of D-galacturonic acid isonicotinyl hydrazone, (*b*) its physical properties, (*c*) its acute toxicity in mice, and (*d*) its antitubercular activity both *in vitro* and *in vivo* against *Mycobacterium tuberculosis* H37Rv.

Isonicotinyl hydrazones derived from other uronic acids, such as D-mannuronic, L-iduronic, and DL-alluronic acids, are being carefully investigated. Both chemical and biological results will be later reported.

EXPERIMENTAL

Preparation of D-Galacturonic Acid Isonicotinyl Hydrazone.—In an Erlenmeyer flask of 1 L. capacity, isonicotinic acid hydrazide (16.7 Gm., product of Pfizer) was dissolved in methyl alcohol (170 cc.) which is acetone free, by heating on the steam bath. To the hot solution, D-galacturonic acid (25 Gm., product from Eastman) dissolved in hot distilled water (150 cc.) was added. The reactants were thoroughly mixed by shaking and the flask heated on the steam bath for ten minutes. Crystalline precipitate (white platelets) was seen to form even when the solution was boiling hot. After twenty-four hours of standing at room temperature, the crystalline product was filtered off with suction, washed first with a small amount of ice-cold distilled water and then with a small amount of methyl alcohol, and finally sucked to dryness. The product was placed in a vacuum desiccator over anhydrous $CaCl_2$ for three days. The weight of the product was 40.5 Gm. (92% of the theoretical yield). On heating, it charred and decomposed between 208 and 210° (uncorr.); 212 and 214° (corr.).

Analysis.—Calcd. for $C_{12}H_{13}O_7N_3$: C, 46.00; H, 4.83; N, 13.42. Found: C, 45.83, 45.91; H, 5.00, 4.92; N, 13.52, 13.56.

Purification.—In contrast to D-glucuronolactone isonicotinyl hydrazone (3) which cannot be recrystallized from an aqueous solution, D-galacturonic acid isonicotinyl hydrazone can be readily purified by recrystallization from boiling water in the presence of a small amount of decolorizing charcoal (Norite). For every 10 Gm. of the hydrazone, 500 cc. of boiling distilled water and 1 Gm. of Norite were used. The hot solution was rapidly

* Received March 12, 1953, from the University of California College of Pharmacy, San Francisco, and the School of Veterinary Medicine, Davis, California, March 9, 1953.

† Professor and Head of Division of Pharmacology, U. C. School of Veterinary Medicine, Davis, California.

The authors are grateful to the staff of the Lilly Research Laboratories (notably Dr. E. C. Kleiderer, Executive Director of Development, and Mr. W. B. Sutton) for performing the biological tests of the new drug against *Mycobacterium tuberculosis* H37Rv, and to Mr. C. Brown, Mr. A. Huitric and Prof. W. D. Kumler for measuring the optical rotation.

TABLE I.—IN VIVO ANTITUBERCULAR ACTIVITY OF D-GALACTURONIC ACID ISONICOTINYL HYDRAZONE IN MICE INFECTED WITH MYCOBACTERIUM TUBERCULOSIS H37Rv

Compound Tested	Dosage	No. of Mice	Days on Test	Per Cent Mortality	Mean Survival Time in Days
D-Galacturonic acid isonicotinyl hydrazone	0.023% in diet	10	28	0	28+
Streptomycin	3000 units weekly by s. c. inj.	10	..	20	27.66+
Control		10	..	90	18.34+

filtered off with suction. The filtrate was allowed to stand in the refrigerator for twenty-four hours. The crystals were filtered off with suction, washed with a small amount of ice-cold methyl alcohol, and sucked to dryness. The purified product was then dried in a vacuum desiccator over anhydrous $CaCl_2$ for three days. Yield: 7.5 Gm.

Analysis.—Found: C, 46.02; H, 4.79; N, 13.45.

From the analytical data, it is apparent that the unrecrystallized, initial product is almost as pure as the recrystallized.

Physical Properties.—Pure D-galacturonic acid isonicotinyl hydrazone prepared and purified by the above procedures forms small, white rods or platelets from boiling water, charring and decomposing between 209 and 210° (uncorr.); 213 and 214° (corr.). It is slightly soluble in cold water but soluble in the boiling solvent (100 cc. distilled water dissolves about 2.2 Gm. of the product at 100° and 0.2 Gm. at 25°). In 5% $NaHCO_3$, 10% NaOH, or 10% HCl solution, it is freely soluble. In sodium bicarbonate solution, it may be used for injection in animals either intravenously or intraperitoneally.

Optical Activity.—Because the solubility of D-galacturonic acid isonicotinyl hydrazone in water at room temperature is rather low, the dilute solution offers difficulties when it is examined for its optical rotation. However, a 2% solution of the hydrazone in 0.2 *N* HCl at 25° can be prepared very easily. When such a solution is examined right away in the polarimeter, it shows an initial rotation $(\alpha)_D^{25}$ of 25.4°. This rotation changes from time to time till it reaches a constant value of + 34.0°. If the solution is allowed to stand for 24 hours and then examined, the $(\alpha)_D^{25}$ found is the constant and checkable value of +34.0°. Apparently, D-galacturonic acid isonicotinyl hydrazone, like sugars and their derivatives, shows the phenomenon of mutarotation.

Acute Toxicity.—Dissolved in sodium bicarbonate solution and given intraperitoneally to mice weighing between 20 and 30 Gm., D-galacturonic acid isonicotinyl hydrazone proved to be much less toxic than the parent drug, isonicotinic acid hydrazide. At a dosage of 1 Gm./Kg. it did not kill any of the mice tested. At a dosage of 1.5 Gm./Kg. it killed only 10% of the mice tested while at 2.0 Gm./Kg., the mortality was 80%. The LD_{50} of D-galacturonic acid isonicotinyl hydrazone must be about 1.8 Gm./Kg./mice (12). Isonicotinic acid hydrazide, according to Pan (6), has a LD_{50} of 150 to 165/Kg./mice, given intravenously or subcutaneously. D-Glucose isonicotinyl hydrazone, according to Grunberg and Schnitzer (7) has a LD_{50} for mice of 1.75 Gm./Kg. by gavage and more than 2.5 Gm/Kg. by subcutaneous injection.

Antitubercular Activity in Vitro and in Vivo.—The biological tests were performed by W. B. Sutton of the Lilly Research Laboratories, Indianapolis, Indiana. *In vitro* tests showed that at a concentration of 0.0002 mg. per cc. of culture, D-galacturonic acid isonicotinyl hydrazone caused practically complete inhibition of growth of *Mycobacterium tuberculosis* H37Rv. *In vivo* tests indicated that at a dosage of 0.023% in the diet given to mice infected with the above mentioned microorganism, the chemotherapeutic effectiveness of this new drug was comparable to or even more favorable than the standard, streptomycin, given in 3,000 units weekly by subcutaneous injection (see the tabulated data above).

SUMMARY

1. A procedure for the preparation of D-galacturonic acid isonicotinyl hydrazone is described.

2. The new drug is found to be much less toxic than isonicotinic acid hydrazide.

3. The new drug possesses very high activity both *in vitro* and *in vivo* against *Mycobacterium tuberculosis* H37Rv.

REFERENCES

(1) The Merck Index, 6th ed. Merck and Co., Inc., Rahway, N. J., (1952), p. 465.
(2) Martin, G. J., *et al.*, *Am. J. Hyg.*, **34***D*, (2), 23(1941); *J. Biol. Chem.*, **139**, 871(1941); *Arch. Intern. Med.*, **69**, 662 (1942); *Ann. Intern. Med.*, **18**, 57(1943); *J. Biol. Chem.*, **151**, 417(1943); British Pat., 564,154 (Sept. 15, 1944); U. S. Pat. 2,366,742 (Jan. 9, 1945).
(3) Sah, Peter P. T., *J. Am. Chem. Soc.*, **75**, 2512(1953).
(4) Unpublished data of W. B. Sutton, The Lilly Research Laboratories, Indianapolis, Indiana and E. G. Roberts, Stanford University School of Medicine, San Francisco, California.
(5) Grunberg, E., and Schnitzer, R. J., *Quart. Bull. Sea View Hosp.*, **13**, 3(1952).
(6) Pan, S. Y., Markaroglu, L., and Reilly, J., *Am. Rev. Tuberc.*, **66**, 100(1952).
(7) Grunberg, E., and Schnitzer, R. J., *Yale J. Biol. Med.*, **24**, 359(1952).
(8) Bernstein, J., Lott, W. A., Steinberg, B. A., and Yale, H. L., *Am. Rev. Tuberc.*, **65**, 357(1952).
(9) Bernstein, J., Jambor, W. P., Lott, W. A., Pansy, F., Steinberg, B. A., and Yale, H. L., *ibid.*, **67**, 363(1953).
(10) Yale, H. L., *et al.*, *J. Am. Chem. Soc.*, **75**, 1935(1953)
(11) Sah, Peter, P. T., and Fan, H. Y., *J. Chinese Chem. Soc.*, **5**, 129(1937).
(12) DeBeer, E. J., *J. Pharmacol. Exptl. Therap.*, **85**, 1(1945).

Tolerance to Bacterial Pyrogens in the Rabbit*

By DAVID M. TENNENT AND WALTHER H. OTT

A study has been made of the development of tolerance to bacterial pyrogens in rabbits, which were given a range of pyrogen doses sufficient to cause temperature rises from 0.22 to 2.49°. A practical means for minimizing tolerance in assay colonies is presented.

THE DEVELOPMENT of tolerance in rabbits given repeated injections of bacterial pyrogen was clearly shown in 1943 by McClosky, Price, Van Winkle, Welch, and Calvery (1). Since that time a number of its aspects have been studied in detail (2–9) but its deleterious effect on the results of pyrogen assays has been generally overlooked.

We report here the results of experiments, interpreted from the standpoint of the routine pyrogen assay, in which the influence of the size and frequency of dosing on the development of tolerance and on the recovery of normal response was studied in rabbits given, at daily, semiweekly, and weekly intervals, sufficient pyrogen to cause initial mean maximum temperature rises of 0.22 to 2.49°.

EXPERIMENTAL

The three hundred seventy-seven rabbits used, taken from our routine assay colony, varied in age and length of service. They had seldom been injected with highly pyrogenic material, but they may not have been free from tolerance. The procedure for their special selection and the techniques for temperature measurement have been described by Tennent and Ott (10).

Five experiments were carried out at different times, with three different samples of bacterial pyrogen, all prepared from *Salmonella newport*.[1] Sample *A* was a liquid culture filtrate precipitated with ammonium sulfate; sample *B* was an agar-washing supernatant precipitated with ammonium sulfate; and sample *C* was agar-washing supernatant precipitated with alcohol and diluted several thousand-fold with solid sodium chloride for convenience in weighing.

Experiment 1.—Preparation *A* was given to 8 groups of 5 rabbits each at 1 μg. per Kg., and to 6 groups of 5 rabbits each at 0.1 μg. per Kg. every day for seven to eleven days. To avoid the influence of environmental factors the individual groups were run consecutively, so that the last day for a particular group was the first day for another.

As shown in Tables I and II, the mean maximum temperature rises decreased rapidly at first and then more slowly, reaching minimum values of half to two-thirds of the initial responses on the fifth, sixth, or seventh days. Thereafter the responses increased somewhat as dosing was continued, but approached the initial values in only 2 of the 14 groups.

Experiment 2.—Two groups of 18 and 19 rabbits respectively were given 2.10 μg. of preparation *B* per Kg. The interval between the first 2 doses was six days, and the animals were dosed twice a week thereafter. As shown in Table III, the responses dropped to 68 and 66% of the initial values on the sixth day and to minima of 36 and 42% on the twenty-third day. Thereafter the responses again increased but did not approach the initial values.

Experiment 3.—Eight groups, each containing 5 rabbits that had been in the colony for one to three and one-half years, were dosed twice weekly with 0.24, 0.41, 0.70, or 1.2 mg. of preparation *C* per Kg. The responses, presented in Table IV, decreased erratically to uniformly low values on the tenth day even though the initial response of one group was only 0.22°

Experiment 4.—Eight groups, each containing 5 rabbits that had been in the colony no longer than six months, were given 2 doses, one week apart, of the same amounts of preparation *C* used in Experiment 3. As shown in Table V, tolerance developed in all groups. Although the initial responses here were significantly higher than those of the rabbits in Experiment 3, which had seen longer service in the colony and were probably somewhat more tolerant at the start, the responses on the seventh day were quite similar.

Experiment 5.—In the first four experiments all of the doses of pyrogen given to any one group of rabbits were always the same size. The following experiment was run to see what would happen when the initial and challenge doses were of different size.

Two groups of 94 rabbits each were given 2 doses of preparation *C*, 1.25 mg. per Kg. and 6.25 mg. per Kg., one week apart. Group I was given the larger dose first (response = 0.96°), and the smaller second (response = 0.41°). Group II was given the smaller dose first (response = 0.61°) and the larger second (response = 0.65°).

Tolerance developed in both groups. The response to the larger dose was 0.31° lower when it was given second than when it was given first (0.96–0.65°), and the response to the smaller dose was 0.20° lower when it was given second than when it was given first (0.61–0.41°). For the larger dose the ratio of the responses was $\frac{0.65}{0.96} = 0.68$, and for the smaller, $\frac{0.41}{0.61} = 0.67$.

Recovery from Tolerance.—All of the rabbits in the first four experiments were used for a study of recovery. Each was allowed to rest for a given period of time and then challenged with a dose the

* Received February 24, 1953, from the Merck Institute for Therapeutic Research, Rahway, N. J.

[1] We are indebted to Dr. Philip R. Edwards, Communicable Disease Center, Public Health Service, for the identification of this organism.

TABLE I.—MEAN MAXIMUM TEMPERATURE RISES OF GROUPS OF 5 RABBITS DOSED DAILY WITH PYROGEN "A," 1 μG. PER KG.

Days After Initial Dose	Temperature Rises, °C.								
	A	B	C	D	E	F	G	H	Average
0	2.17	1.51	1.76	2.02	2.49	1.92	1.67	2.19	1.97
1	2.13	1.49	1.66	2.16	1.62	1.53	1.76	1.95	1.79
2	2.08	1.45	1.41	1.81	1.52	1.53	1.48	1.80	1.63
3	1.62	1.10	1.34	1.43	1.80	1.58	1.60	1.79	1.53
4	1.45	1.04	1.34	1.38	1.04	1.22	1.60	1.62	1.34
5	1.30	1.35	1.16	1.09	1.24	1.40	1.00	1.24	1.22
6	1.12	0.90	1.18	1.15	1.06	1.05	1.15	1.12	1.09
7	1.39	1.27	0.88	0.89	0.78	0.69	...	...	0.98
8	1.25	1.14	...	...	0.79	0.96	...	...	1.03
9	1.20	1.21	...	...	1.32	1.22	...	...	1.24
10	...	...	...	...	1.30	1.26	...	...	1.28

TABLE II.—MEAN MAXIMUM TEMPERATURE RISES OF GROUPS OF 5 RABBITS DOSED DAILY WITH PYROGEN "A," 0.1 μG. PER KG.

Days After Initial Dose	Temperature Rises						
	A	B	C	D	E	F	Average
0	1.75	1.75	1.04	1.09	1.26	1.31	1.37
1	0.99	1.21	1.12	0.90	1.28	1.03	1.09
2	0.61	0.84	1.19	1.12	1.18	0.85	0.97
3	0.85	0.92	0.78	1.15	1.09	1.05	0.97
4	1.08	1.30	0.92	1.20	1.05	1.09	1.11
5	0.72	0.91	0.05	0.53	0.60	0.60	0.57
6	0.75	1.48	0.68	0.60	1.04	1.10	0.94
7	0.48	0.32	1.02	0.66	...	...	0.62
8	...	...	0.90	0.85	...	...	0.88
9	...	...	1.11	1.18	...	...	1.15
10	...	...	0.91	0.87	...	...	0.89

same size as the tolerance producing dose. As shown in Table VI the variation in rate of recovery was large. None of the rabbits challenged after seven days had recovered appreciably. Of the 14 groups challenged after fourteen days, 4 had recovered less than 50% of the lost response and 3 more than 100%. Of the 12 groups challenged after twenty-one days 6 had recovered less than 50% and 2 more than 100%.

DISCUSSION

In the experiments reported here, tolerance developed in each of the 30 groups of rabbits used regardless of the size of the pyrogen dose. Of 6 groups with initial responses between 0.6 and 0.9°, (Table IV, 1.2 mg. per Kg.; Table V, 0.41 and 0.70 mg. per Kg.) 5 gave subsequent responses to the same dose of less than 0.6, and 4 of these were less than 0.4°. Thus many pyrogenic samples may not be detected when tolerant rabbits are present in the colony.

It is difficult to say what the relation is between the size of the dose and the tolerance it produces. If we express tolerance as the difference between the expected temperature rise and the observed temperature rise after a given dose, the data in Experiments 1–5 show that in general a large dose will cause more tolerance than will a small dose. This does not seem to be a fair comparison, however, because the decrease in response in rabbits given large doses can be great, but in rabbits given small doses it must be small. In fact the loss of response of the groups in Tables I, II, and III exceeded the initial responses of a number of the groups in Tables IV and V.

If we express the tolerance produced as the ratio of the responses to the final and initial doses then the results of Experiments 1, 3, and 4, as shown in Fig. 1, do not indicate a relation between the size of the dose and the tolerance produced. The results of Experiment 5 indicate again that on this basis the size of the dose is not a major factor, since the tolerance ratio was the same when the initial and challenge doses were of different size.

The frequency of dosing apparently has little effect on the initial development of tolerance, as shown by the results of Experiments 1, 3, and 4, pre-

TABLE III. MEAN MAXIMUM TEMPERATURE RISES OF GROUPS OF RABBITS DOSED REPEATEDLY WITH PYROGEN "B," 2.1 μG. PER KG.

Days After Initial Dose	Temperature Rises ° C	
	Group *A* 18 Rabbits	Group *B* 19 Rabbits
0	1.68	1.78
6	1.14	1.18
9	1.00	1.20
13	1.00	1.16
16	1.02	1.01
20	1.01	1.12
23	0.61	0.74
27	0.88	0.96
30	0.99	1.08
37	1.21	1.26
42	0.96	...

TABLE IV.—MEAN MAXIMUM RESPONSES OF GROUPS OF 5 RABBITS DOSED TWICE WEEKLY WITH PYROGEN "C"

Days After Initial Dose	Temperature Rises of Groups Following Doses Specified							
	0.24 Mg./Kg., °C.		0.41 Mg./Kg., °C.		0.70 Mg./Kg., °C.		1.2 Mg./Kg., °C.	
0	0.24	0.22	0.49	0.47	0.56	0.54	0.78	0.81
3	0.25	0.26	0.37	0.42	0.27	0.21	0.70	0.67
7	0.22	0.19	0.21	0.22	0.35	0.26	0.52	0.72
10	0.05	0.08	0.16	0.18	0.24	0.24	0.49	0.44

TABLE V.—MEAN MAXIMUM RESPONSES OF GROUPS OF 5 RABBITS GIVEN TWO DOSES OF PYROGEN "C" ONE WEEK APART

Days After Initial Dose	Temperature Rises of Groups Following Doses Specified							
	0.24 Mg./Kg., °C.		0.41 Mg. Kg., °C.		0.70 Mg./Kg., °C.		1.2 Mg./Kg., °C.	
0	0.40	0.43	0.69	0.67	0.82	0.87	1.07	1.05
7	0.26	0.25	0.34	0.33	0.35	0.37	0.70	0.65

TABLE VI.—RECOVERY FROM TOLERANCE

The first column shows the mean maximum temperature rise of groups of 5 rabbits the first time they received given doses of pyrogen.

The second column shows the number of tolerance-producing doses given; the third, the interval between these doses and the fourth, the response to the last of these doses.

The fifth column shows the number of days of rest allowed before the challenge dose, which was the same size as the tolerance-producing doses in each case; the sixth column shows the response to the challenge dose; and the seventh, the per cent recovery of the lost response, calculated as indicated above the column headings.

A, Initial Response	No. of Doses	Frequency of Dosage	B, Tolerant Response	Days of Rest	C, Response to Challenge	$\frac{C-B}{A-B} \times 100$ Per Cent Recovery
2.17	10	Daily	1.20	7	1.24	4.2
1.51	10	Daily	1.21	12	1.15	−20.0
1.76	8	Daily	0.88	14	1.54	75.0
2.02	8	Daily	0.89	21	1.94	92.9
2.49	11	Daily	1.30	7	1.30	0.0
1.92	11	Daily	1.26	14	1.70	66.7
1.67	7	Daily	1.15	14	1.80	125.0
2.19	7	Daily	1.12	21	1.26	13.1
1.75	8	Daily	0.48	14	1.26	61.4
1.75	8	Daily	0.32	21	1.38	74.2
1.04	11	Daily	0.91	7	0.74	−130.9
1.09	11	Daily	0.87	14	1.02	68.2
1.26	7	Daily	1.04	14	1.79	340.5
1.31	7	Daily	1.10	21	1.16	28.5
0.40	2	Weekly	0.26	14	0.27	7.2
0.43	2	Weekly	0.25	21	0.40	83.4
0.69	2	Weekly	0.34	14	0.45	31.2
0.67	2	Weekly	0.33	21	0.49	47.1
0.82	2	Weekly	0.35	14	0.60	53.2
0.87	2	Weekly	0.37	21	0.51	28.0
1.07	2	Weekly	0.70	14	0.81	29.7
1.05	2	Weekly	0.65	21	0.66	2.5
0.24	4	2×/week	0.05	14	0.26	110.4
0.22	4	2×/week	0.08	21	0.39	221.5
0.49	4	2×/week	0.16	14	0.39	69.7
0.47	4	2×/week	0.18	21	0.29	37.9
0.56	4	2×/week	0.24	14	0.43	59.4
0.54	4	2×/week	0.24	21	0.41	56.7
0.78	4	2×/week	0.49	14	0.56	24.1
0.81	4	2×/week	0.44	21	0.84	108.0

sented in Fig. 2. The onset of tolerance is provoked by a single dose, after which the rabbit becomes progressively more tolerant during the next week. If additional doses are given during this time the final tolerance may be greater at a later date, and recovery may be delayed, but the status on the seventh day will not be significantly influenced.

A theoretical curve illustrating the course of tolerance in a rabbit given pyrogen is shown in Fig. 3. The plotted points are composite data from Tables I to VI. Recovery from tolerance begins near the ninth day after the last pyrogen dose. While, as found by others (1, 2), the average time for complete recovery was about three weeks, the variation was great. Some rabbits recovered in fourteen days, and others were far from recovery at twenty-one days. We do not have enough data here to determine the influence of the size of the dose or the number of previous doses on the recovery rate, but recovery does not seem to be more rapid after a small dose than after a large one.

Our experience in routine testing and the results of these experiments show that the effect of tolerance in animals which have received pyrogen will not be

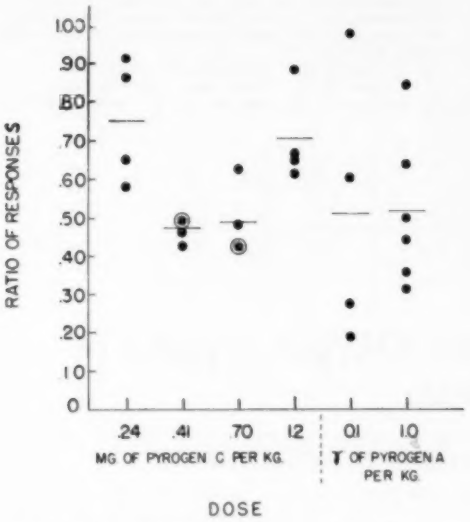


Fig. 1.—Comparison of the ratios of the responses on the initial and seventh days in Experiments 1, 3, and 4, with the sizes of the doses.

avoided by the usual three- or four-day rest period between tests, and that a longer time is required. However, if every rabbit must be rested for three or four weeks between tests the size of the colony necessary for control testing will be increased several-fold, and pyrogen assays will become considerably more expensive than they are now.

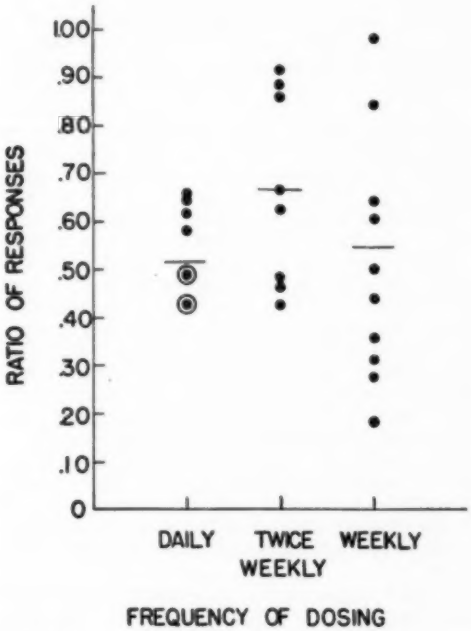


Fig. 2.—Comparison of the ratios of the responses on the initial and seventh days in Experiments 1, 3, and 4 with the frequency of dosing.

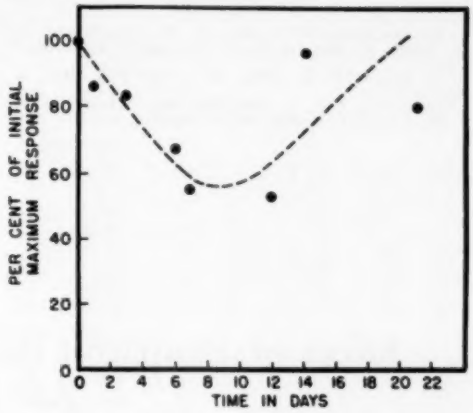


Fig. 3.—The development of and recovery from tolerance to bacterial pyrogen in the rabbit.

The downward portion of the curve illustrates the development of tolerance resulting from a single dose of pyrogen. The upward portion illustrates the recovery after the last of a series of doses.

While this dilemma can be resolved only by further research, we do have a practical compromise, that appears to give reasonably reliable results. We assign each rabbit an extra day of rest for each 0.1° temperature rise in its last test. Since our colony rabbits are normally taken in rotation, the average rest period usually works out to be somewhat longer than this. If the previous temperature rise was between 0.8 and 1.4°, the rabbit gets a rest of about two weeks. If it was between 1.5 and 2.1°, the rest is about three weeks. By the time that this schedule had been in use for four months, the slope of the dose response relation of our colony had returned from a low value of 0.3 (°C. *vs.* log. dose) to the initially determined 0.78. The latter value for slope may not be characteristic of the response of nontolerant rabbits, but it is at least high enough to be useful.

SUMMARY

1. Tolerance to bacterial pyrogens developed in all rabbits given a range of pyrogen doses sufficient to cause temperature rises that varied from 0.22° to 2.49°.

2. The development of tolerance was initiated by a single dose of pyrogen, after which the rabbits became progressively more tolerant during the next week. Additional dosing during this period had little influence on the rate of development.

3. The average time for recovery was about three weeks after the last dose, but the variation about this point was great.

4. The use of tolerant rabbits in assay colonies may result in failure to detect pyrogenic samples.

5. A practical means for minimizing tolerance in assay colonies is presented.

REFERENCES

(1) McClosky, W. T., Price, C. W., Van Winkle, W., Jr., Welch, H., and Calvery, H. O., THIS JOURNAL, **32**, 69 (1943).
(2) Beeson, P. B., *Proc. Soc. Expll. Biol. Med.*, **61**, 248(1946).
(3) Beeson, P. B., *J. Expll. Med.*, **86**, 29(1947).
(4) Beeson, P. B., *ibid.*, **86**, 39(1947).
(5) Bennett, I. L., Jr., *ibid.*, **88**, 267(1948).
(6) Heyman, A., *Venereal Disease Inform.*, **26**, 51(1945).
(7) Favorite, G. O., and Morgan, H. R., *J. Lab. Clin. Med.*, **31**, 672(1946).
(8) Morgan, H. R., *J. Immunol.*, **59**, 129(1948).
(9) Morgan, H. R., *J. Clin. Invest.*, **27**, 706(1948).
(10) Tennent, D. M., and Ott, W. H., *Analyst*, **77**, 643 (1952).

Effect of Panparnit on the Oxygen Uptake of Rat Brain*

By LASLO KAJDI and CLARA H. WILLIAMS

The effect of the anticonvulsant drug Panparnit on the *in vitro* oxygen consumption of slices from different areas of rat brain was studied. An inhibition resulted which was selective for the cortical slices in lower concentrations. The action of three convulsant drugs on the oxygen metabolism of brain slices was studied; these also produced an inhibition. Possible explanations of this inhibition are discussed.

In 1950, Himwich (1) reported that several antiepileptic drugs, including trimethyloxazolidine-dione, were capable of restoring to normal the epileptic brain waves produced by diisopropyl fluorophosphate (DFP) in curarized rabbits. Essig and Himwich (2) further reported that Panparnit hydrochloride[1] (the diethylamino ethyl ester of 1-phenyl-cyclopentane-1-carboxylic acid) also restored the abnormal brain waves produced by DFP.

Richards and Taylor (3) found that the anticonvulsive drug, trimethyloxazolidine-dione, produced an inhibition of the oxygen uptake of mouse cortex slices and of mouse brain brei in both glucose and pyruvate substrate solutions.

The purpose of the present experiments was to determine whether Panparnit, having an action similar to trimethyloxazolidine-dione in the rabbit experiments of Himwich, would affect the oxygen metabolism of rat brain in the same way that trimethyloxazolidine-dione did the mouse brain metabolism.

* Received April 24, 1953, from the Research Laboratory, Veterans Administration Hospital, Perry Point, Md.
Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.
[1] Panparnit hydrochloride was supplied by Geigy Pharmaceuticals through the kindness of Dr. V. Froelicher.

MATERIALS AND METHODS

Oxygen consumption of brain slices and brain brei was measured by the Warburg technique. White rats of either sex were decapitated with large shears, the brains quickly removed, sliced and weighed on a torsion balance. The slices were transferred immediately to the reaction vessels which contained 2 ml. of Krebs-Ringer phosphate and either 1 ml. of 0.1 *M* dextrose solution or 1 ml. 0.02 *M* pyruvate. The center well contained 0.2 ml. of 10% potassium hydroxide solution. Calculated amounts of the drugs were dissolved in the Krebs-Ringer phosphate solution just before use. The *p*H of the resulting solutions was adjusted to 7.3. The flasks were aerated with pure oxygen for four minutes, placed in the constant temperature bath kept at 37.5°, and equilibrated for ten minutes.

Each hemisphere of the brain was cut into 6 longitudinal slices and two consecutive slices were used per flask. The slices of one hemisphere served as controls being placed in the buffer solution while those of the other hemisphere were placed in flasks containing the drug being tested in the buffer solution. The first 2 slices consisting chiefly of gray matter, called cortical slices, were placed in the first flask; the next two consisting chiefly of white matter, called white slices, were placed in the second flask; and the last two of basal ganglia, called ganglia slices, were placed in the third flask. The combined weight of the slices in the individual flasks ranged from 90 mg. to 230 mg. Three transverse slices were made from the cerebellum, the total weight of which averaged about 180 mg.

In experiments using brain brei, the whole brain was weighed, homogenized in 2 ml. Krebs-Ringer phosphate solution and made up to a final volume of 6 ml. One ml. aliquots of this mixture containing about 150 mg. of brain substance were added to each flask.

Oxygen consumption (Q_{O_2}) is expressed in microliters per mg. of fresh weight per hour. No moisture was added to the slices. Weighings were made quickly, so that there was no loss of weight through evaporation.

TABLE I.—EFFECT OF VARIOUS CONCENTRATIONS OF PANPARNIT ON THE OXYGEN CONSUMPTION OF RAT BRAIN SLICES

Concentration of Panparnit, mM/L.	Cortical Slices Q_{O_2} Exper.	Cortical Slices Q_{O_2} Control (No Panp.)	Cortical Slices Inhib., %	White Slices Q_{O_2} Exper.	White Slices Q_{O_2} Control (No Panp.)	White Slices Inhib., %	Ganglia Slices Q_{O_2} Exper.	Ganglia Slices Q_{O_2} Control (No Panp.)	Ganglia Slices Inhib., %	Cerebellum Q_{O_2} Exper.	Cerebellum Q_{O_2} Control (No Panp.)	Cerebellum Inhib., %
0.1 *M* Dextrose as Substrate												
0.41	1.75	1.99	12	1.92	2.02	5	1.62	1.64	0	2.07	1.97[a]	0
0.41	1.36	1.88	27	1.83	1.83	0	...	...	..	1.90	1.97	0
0.51	1.07	2.10	49	1.66	1.82	9	1.68	1.87	10	1.87	1.97	5
0.70	0.54	1.75	69	1.23	1.74	29	1.20	1.75	31	1.55	1.97	21
0.83[b]	1.02	2.12	52	1.14	1.84	38	0.99	1.86	46	1.23	1.97	37
1.03	0.41	1.68	75	0.84	1.98	58	1.17	1.74	32	0.96	1.97	52
5.3	0.19	1.85	90	0.27	1.65	84	0.28	1.46	81	0.26	1.97	87
10.3	0.21	1.74	88	0.17	1.45	88	0.12	1.51	92	0.17	1.97	92
0.02 *M* Sodium Pyruvate as Substrate												
0.41	1.07	2.42	55	1.56	2.09	25	1.41	1.92	26	1.43	1.99	28
0.62	0.70	2.25	69	0.97	2.31	58	0.99	1.86	46	0.90	1.99	55
1.03	0.41	2.22	86	0.45	1.94	77	0.50	1.70	70	0.90	1.99	55

[a] Based upon 9 previous determinations with normal cerebellum in Ringer-phosphate and dextrose.
[b] Cortex in this case was scraped off and used in place of cortical slices; other slices thus contained no cortex at all.

The per cent inhibition of the oxygen consumption was calculated by the formula:

$$\frac{Q_{O_2}\text{ control} - Q_{O_2}\text{ experimental}}{Q_{O_2}\text{ control}} \times 100 = \%\text{ inhibition}$$

On the basis of 76 preliminary experiments using brain slices with only the Krebs-Ringer solution and dextrose, we found that the variations between corresponding slices of the two hemispheres was less than 10%. Thus in the data presented, only values varying more than 10% from the control are regarded as showing significant differences.

RESULTS

Table I records the effect of Panparnit (added to buffered medium in the Warburg apparatus) on the oxygen consumption of rat brain slices. Both dextrose and pyruvate substrates were used. It will be noted that Panparnit inhibits the Q_{O_2} of the *cortical* brain slices in the range of concentrations between 0.4 m*M* per liter[2] and 1.03 m*M* per liter significantly more than that of the rest of the brain. Above 1.03 m*M* concentration, however, there is a uniform and extreme inhibition of the whole brain.

The Q_{O_2} of the cerebellar slices is also included in Table I and it will be noted that the amount of inhibition by Panparnit corresponded with its action on the noncortical slices of the brain.

In experiments using pyruvate as substrate, the Panparnit inhibited the oxygen consumption to a greater degree than with dextrose as substrate. The selectivity for the cortical slices however, was less marked.

The results of the experiments with brain brei are shown in Table II. The inhibitory action of Panparnit on the Q_{O_2} of the homogenized brain parallels that found with brain slices with both substrates.

We found that Panparnit, in a manner similar to trimethyloxazolidine-dione, inhibited the Q_{O_2} of brain in the Warburg apparatus. The question then arose whether the anticonvulsive action was mediated through its influence on oxygen metabolism. If this were the case one would expect that convulsive drugs would increase the Q_{O_2} of the brain or at least would leave it unaffected.

The action of nikethamide, picrotoxin, and pentylenetetrazol on the oxygen consumption of rat brain slices was therefore studied. Table III illustrates that nikethamide at a 5.8 m*M* concentration inhibited the Q_{O_2} significantly, and a selectively increased inhibition of Q_{O_2} for the cortical slices was noted at this concentration. When the concentration was raised to 11.7 m*M* the inhibition of the Q_{O_2} was more intense, but the same for all areas of the brain.

Picrotoxin from 0.331 m*M* to 0.662 m*M* concentrations showed similarly a selectively greater inhibition of Q_{O_2} of the cortical slices, while at a higher concentration (1.65 m*M*) the inhibition was more intense and uniform for all types of slices.

Pentylenetetrazol at 9.4 m*M* concentration showed a selectively greater inhibition of Q_{O_2} for the cortical slices, while at 20.5 m*M* concentration the inhibition was uniform for all types of brain slices. At this same degree of inhibition (21%) the former two drugs, in contrast, still exhibited the specificity for the cortical slices.

TABLE II.—EFFECT OF VARIOUS CONCENTRATIONS OF PANPARNIT ON THE OXYGEN UPTAKE OF BRAIN BREI[a]

Concentration of Panparnit, mM/L.	Q_{O_2} Experimental	Q_{O_2} Control (No Panparnit)	Inhibition %
0.1 *M* Dextrose as Substrate			
None	...	1.26 (24)[b]	..
0.103	1.18 (3)	1.37 (3)	13
0.41	1.12 (3)	1.44 (3)	22
1.23	0.37 (3)	1.30 (3)	71
0.02 *M* Pyruvate as Substrate			
None	...	1.44 (6)	..
0.103	1.32 (3)	1.71 (3)	22
0.41	0.67 (3)	1.60 (3)	58
0.82	0.25 (3)	1.51 (3)	83

[a] One portion of same brei used as control.
[b] ( ) indicates the number of determinations from which given averages were calculated.

[2] These values and those described subsequently represent "final concentration" of the drug in the buffered medium.

TABLE III.—OXYGEN CONSUMPTION OF RAT BRAIN SLICES IMMERSED IN VARIOUS CONCENTRATIONS OF NIKETHAMIDE, PICROTOXIN, AND PENTYLENETETRAZOL IN RINGER-PHOSPHATE BUFFER AND 0.1 *M* DEXTROSE

Concentration of Drug, *m*M/L.	Cortical Slices Q_{O_2} Exper.	Cortical Slices Q_{O_2} Control (No Drug)	Cortical Slices Inhib., %	White Slices Q_{O_2} Exper.	White Slices Q_{O_2} Control (No Drug)	White Slices Inhib., %	Ganglia Slices Q_{O_2} Exper.	Ganglia Slices Q_{O_2} Control (No Drug)	Ganglia Slices Inhib., %	Cerebellum Q_{O_2} Exper.	Cerebellum Q_{O_2} Control (No Drug)	Cerebellum Inhib., %
Nikethamide												
2.9	1.36	1.53	11	1.67	1.77	6	1.61	1.89	15	1.72	1.97[a]	13
5.8	1.01	1.75	42	1.23	1.81	32	1.26	1.74	28	1.32	1.97	33
11.7	0.75	1.71	56	0.76	1.85	59	0.89	1.81	51	0.96	1.97	51
Picrotoxin												
0.331	1.60	2.00	20	1.56	1.86	16	1.86	1.85	0	2.24	1.97	0
0.662	1.55	2.17	29	1.56	1.81	14	1.87	1.71	0	1.79	1.97	9
1.65	1.07	1.80	40	1.05	1.72	39	1.13	1.65	31	...	...	..
Pentylenetetrazol												
9.4	1.64	2.16	24	1.72	1.77	0	1.95	1.88	0	2.25	1.97	0
9.4	1.42	1.90	25	1.75	1.66	0	1.90	1.91	0	...	...	..
9.4	1.64	1.91	16	1.69	1.82	7	1.71	1.67	0	...	...	..
20.5	1.56	1.90	18	1.57	1.91	18	1.56	2.15	28	1.61	1.97	18
20.5	1.51	1.95	23	1.56	1.98	21	1.65	1.87	12	1.73	1.97	12

[a] Based on 9 previous determinations with normal cerebellum in Ringer-phosphate and dextrose.

DISCUSSION

Our studies have shown that Panparnit has an inhibitory effect on the Q_{O_2} of both rat brain slices and rat brain brei when either glucose or pyruvate was used as substrate. Thus its action is similar to trimethyloxazolidine-dione, also an anticonvulsant. In our experiments, however, we have been able to demonstrate that Panparnit selectively inhibited the *cortical* slices.

Wortis (4) reported that immersion of minced rat brain in a medium containing pentylenetetrazol produced no inhibition of the oxygen uptake. Our experiments did not confirm this observation since the addition of pentylenetetrazol to the buffer solution diminished the oxygen uptake. The two other convulsants which we studied, nikethamide and picrotoxin behaved similarly. Wortis and other previous investigators used either brain brei or cortex slices only. With our technique of comparing slices from different depths of the rat brain, selective inhibition of the *cortical* slices could be demonstrated.

Both the convulsant and anticonvulsant drugs studied by us showed a selective action on the cortex of the brain, but the selectivity became masked as the degree of inhibition increased. Pentylenetetrazol showed this selectivity to a lesser degree than the other convulsant drugs tested.

Caution ought to be taken in interpreting these results as indicating that the cortical brain cells are more sensitive to these drugs than are the cells of the deeper layers of the brain. It is possible that the actions of the drugs on the brain cells of the deeper layers are partially concealed by the diluting effect of the more inert white matter in these sections.

Finding that both convulsant and anticonvulsant drugs influence the Q_{O_2} in the same direction suggests that the action of anticonvulsant drugs may not be mediated solely through the oxygen consumption. Further studies are planned to ascertain whether this inhibitory action of the above drugs on the carbohydrate metabolism of brain is exerted at the same or at different phases of the metabolic cycle. This may be accomplished by using various substrates, auxillary electron transport agents, etc.

Studies of the action of these drugs on the gray substance of medulla oblongata may prove it to be a more sensitive tissue for measuring the *in vitro* action especially of the convulsant drugs.

SUMMARY

1. Inhibition of Q_{O_2} of brain slices and brei was observed with the anticonvulsant, Panparnit, as previously reported in case of trimethyloxazolidine-dione. In the present experiments, however, selective inhibition of the cortical slices in the lower concentrations of the drug was demonstrated.

2. The three convlusant drugs studied inhibited the oxygen consumption. In lower concentration they inhibited the Q_{O_2} of the cortical slices of brain significantly more than that of other portions of the brain. In higher concentration this inhibition was much greater and uniform for the whole brain.

3. Since both convulsant drugs as well as anticonvulsant drugs depress the oxygen consumption of brain, the inhibitory action of these drugs may not be related to their anticonvulsive action and the results do not necessarily bear a relationship to the mechanism of their action *in vivo*.

REFERENCES

(1) Himwich, H. E., Essig, C. F., Hampson, J. L., Bales, P. D., and Freedman, A. M., *Am. J. Psychiatry*, **106**, 816–820(1949–1950).

(2) Essig, C. F., Hampson, J. L., Bales, P. D., Willis, Alice, and Himwich, H. E., *Science*, **111**, 38(1950).

(3) Taylor, J. D., Richards, R. K., Everett, G. M., and Bertcher, Elizabeth L., *J. Pharm. Exptl. Therap.*, **98**, 393 (1950).

(4) Wortis, S. B., *New York State J. Med.*, **38**, 1015 (1938).

The Spectrophotometric Determination of Phenylacetone in Amphetamine*

By STANLEY K. FREEMAN

A rapid and precise method is described for the ultraviolet spectrophotometric determination of phenylacetone in amphetamine. The absorbances of ethanolic solutions of the amine containing up to 10 per cent of ketone are measured at 290 mμ and the amount of phenylacetone present read from a standard curve. There is good correlation between purity of amphetamine samples examined and their phenylacetone content.

IN THE COURSE of an investigation on amphetamine (alpha-methylphenethylamine) it was necessary to determine the amount of an intermediate, phenylacetone, present. Ebstein and van Meter (1) have reported a spectrophotometric procedure for the analysis of amphetamine and its salts in pharmaceutical preparations, but no references have been found in the literature with regard to the determination of impurities occurring in this compound. There are several publications concerned with the ultraviolet absorption spectrum of phenylacetone (2–4). Although ethanol was the solvent employed in all instances, one of the articles (2) reported fine structure in the ketone's spectrum, whereas the remaining two exhibited none. The writer has found that ethanolic solutions of carefully purified phenylacetone evidence fine structure in the region of 260 mμ and a broad band in the neighborhood of 285 mμ. A spectrum similar to that described by Kumler, *et al.* (2), which has fine structure in the "carbonyl" region also, was obtained using isooctane as the solvent. Figure 1 depicts the spectra of phenylacetone in ethanol and isooctane and that of amphetamine in ethanol.

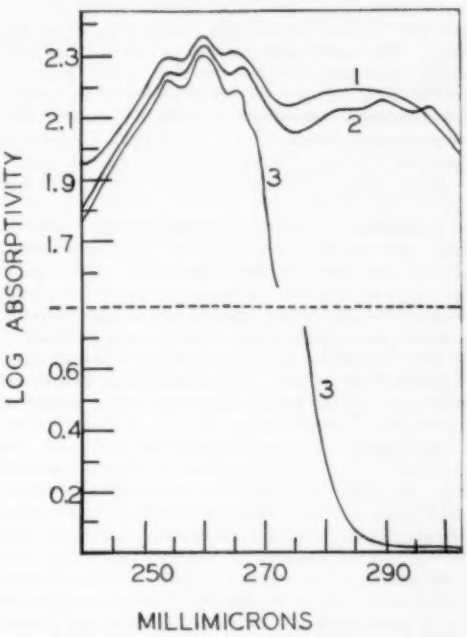


Fig. 1.—Absorbance of phenylacetone solutions in amphetamine: *1*, phenylacetone in ethanol; *2*, phenylacetone in isooctane; *3*, amphetamine in ethanol.

EXPERIMENTAL

To decrease materially the background absorbance of amphetamine at higher concentrations, the wave length at 290 mμ was selected for this investigation. Phenylacetone displays only slightly lower absorbance at this wave length as compared with its 285 mμ maximum. Solutions of phenylacetone in purified amphetamine were examined at 290 mμ and three standard curves obtained in the range between 0 and 10% (Fig. 2). Preliminary study indicated that up to 99% phenylacetone can be determined, but this work was not pursued further since the quantity of the ketone present as an impurity in the amine never exceeded 5%.

In order to meet U. S. P. requirements, amphetamine must be at least 97% pure by acidimetric titration. Several samples of amphetamine prepared from phenylacetone (5) were analyzed by this method, in addition to determining the phenyl acetone content. The results are presented in Table I. One of the nine samples examined (i) failed to meet the assay minimum and exhibited a much higher ketone content than the other materials listed. The standard deviation for the spectrophotometric procedure is in the order of 0.15%.

Reagents.—Phenylacetone was prepared from benzyl cyanide by the method of Bobranski and Drabik (5). Purification was effected by several fractionations through a $2^1/_2$-ft. Widmer column followed by conversion to the semicarbazone which was recrystallized three times from ethanol (m. p. 188.0–188.5°). The ketone was regenerated with 10% phosphoric acid, the aqueous solution extracted with ether and the water white phenylacetone fractionated. The final product distilled at 87° and 7 mm. Titration of the acid liberated by reacting the ketone with hydroxylamine hydrochloride yielded purity values of 99.78% and 99.85%.

* Received December 1, 1952, from the Benzol Products Company, Newark, New Jersey.

TABLE I.—CORRELATION BETWEEN PURITY OF AMPHETAMINE AND PHENYLACETONE CONTENT

Sample	Phenylacetone, %	Purity, %
a	0.10	99.60
b	0.19	98.85
c	0.31	98.58
d	0.70	98.50
e	0.82	97.96
f	0.93	98.25
g	0.95	98.25
h	1.60	97.60
i	3.21	96.13

Amphetamine was synthesized from phenylacetone (5) and purified by conversion to the sulfate. The sulfate salt was recrystallized twice from 60% ethanol-water and the free amine regained in the usual manner (1). The purity of the resulting compound was found to be 99.79% and 99.88% when assayed by the U. S. P. procedure and 99.60% and 99.78% by a Kjeldahl nitrogen determination.

Ethyl alcohol (SD 30, U. S. Ind. Chem. Co.) was used as the solvent.

Apparatus.—The apparatus consisted of a Beckman spectrophotometer, Model DU, fused silica absorption cells, 1-cm. light path. Absorption spectra were obtained by recording absorbance readings at 2 mμ intervals, and, in the neighborhood of maxima and minima, the interval was reduced to 1 mμ. Constant slit widths were maintained in regions of all maxima. The individual points are not shown on the graphs since they are so numerous as to detract from clarity.

Procedure.—The procedure for preparing the standard curves is exemplified by the *B* curve in Fig. 2 (10% amphetamine solution) as follows. Add from a 10-ml. burette a volume of purified phenylacetone solution (ethanolic) corresponding to 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.10 Gm. each of the ketone to ten 100-ml. volumetric flasks. Sufficient ethanol is added to bring the volume to 10 ml. and the flask then diluted to the mark with an 11.1% solution of amphetamine in ethanol. The absorbance as measured against an ethanol blank is plotted *versus* weight of phenylacetone taken for the analysis. A reading at zero per cent phenylacetone is also recorded. Three absorbance readings are taken for every dilution and must agree within 0.003. The slit width selected for this work was 0.7 mm. The terms absorbance and absorptivity are in conformity with the list published by the Society for Applied Spectroscopy (6) and are defined as follows:

$$t = I/I_0$$
$$-\log_{10} t = A = abc$$

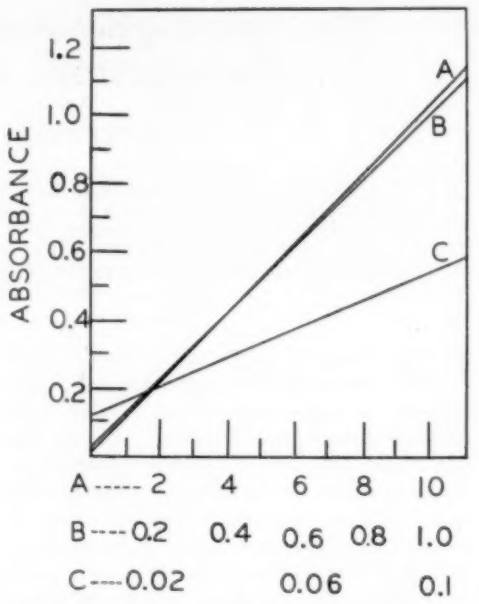


Fig. 2.—Per cent phenylacetone in amphetamine sample: *A*, 1% amphetamine sample in ethanol; *B*, 10% amphetamine sample in ethanol; *C*, 50% amphetamine sample in ethanol.

where *t* is the transmittance, *A* the absorbance, and *a* the absorptivity.

Pipette 10 ml. of the amphetamine sample into a 100-ml. volumetric flask, dilute to the mark with ethanol and transfer a portion of the well-mixed solution to a 1-cm. fused silica absorption cell. The absorbance is measured at 290 mμ against an ethanol blank. If the reading is below 0.100 or above 1.25, a new solution is prepared using either 1 ml. or 50 ml. of the amphetamine sample, and the process repeated. The per cent phenylacetone in the material is then read from the appropriate standard curve.

REFERENCES

(1) Ebstein, I., and van Meter, C. T., THIS JOURNAL, **41**, 372(1952).
(2) Kumler, W. D., Strait, L. A., and Alpen, E. L., *J. Am. Chem. Soc.*, **72**, 1463(1950).
(3) Ramart-Lucas, and Guerlain, J.-P., *Bull. soc. chim.*, **49**, 1860(1931).
(4) Ramart-Lucas, and La Baune, L., *Ann. chim.* **16**, 276 (1931).
(5) Brobranski, B. R., and Drabik, Ya. V., *J. Applied Chem.* (U. S. S. R.), **14**, 410(1941).
(6) Society for Applied Spectroscopy, Bull. 4, No. 2 (1949).

Tolerance, Toxicity, and Caloric Availability of Intravenously Injected Oxypolygelatin Solution in Rabbits*

By LAWRENCE E. DETRICK, VIRGINIA DEBLEY, and THOMAS J. HALEY

Prolonged and repeated intravenous injections of oxypolygelatin in large volumes, even though tolerated as shown by a low mortality ratio, produced physical deterioration and illness in rabbits. Hydropic degeneration occurred in the convoluted tubule epithelium and was not reversible fourteen days after injections were stopped. The glomeruli of the kidney and the endothelium of vessels in the lungs were not involved. Foam cells were not observed in the reticuloendothelial system of injected animals. The toxic effects of intravenously injected oxypolygelatin appear to be dependent upon characteristics of the material injected other than the presence of small molecular particles in a polydisperse OPG system.

OXYPOLYGELATIN (OPG), an oxidized, polymerized gelatin, was introduced by Pauling, *et al.* (1), and Campbell, *et al.* (2), as a plasma volume expander. Their preparations were tolerated by mice, rabbits, and dogs; were nonantigenic in guinea pigs and rabbits; were retained in hemorrhaged rabbits to the extent of 25–50 per cent for six hours, and were well tolerated by humans in a limited number of clinical trials (2). However, initial (3) and subsequent (4) OPG preparations, when injected into rats, produced extensive morphological kidney degeneration accompanied by polyuria and azotemia. Lippman, *et al.* (4), postulated the toxicity and proteinuria to be the result of tubular epithelial saturation with small OPG molecules, since oxypolygelatin preparations to date are polydisperse systems with wide variation in molecular size. Millican, *et al.* (5), found that normal mice manifested a toxicity to intravenously infused OPG solution when given in volumes equivalent to 5–15 per cent of the body weight.

Although introduced in 1944 (1), extensive investigations of intravenously injected OPG has been limited by its comparative unavailability for study (6). Oxypolygelatin has been administered to animals under various experimental conditions—hemorrhagic shock (7); following moderate and severe hemorrhage (8–10); after scald burns (11, 12); for its effect on renal function (13), and in an evaluation of its conversion to glucose (14, 15). Inasmuch as these investigations contributed only comparative mortality data and nothing concerning chronic toxicity or caloric availability, we have studied the effect of prolonged and repeated intravenous injections of large volumes of OPG. Observations made in the present experiment have been compared with results previously reported (16) on rabbits similarly injected with tapioca dextrin solution.

EXPERIMENTAL

Female Dutch strain rabbits averaging 1,925 Gm. body weight were divided into 5 experimental groups (Table I). Intravenously infused animals received 11 consecutive daily injections administered via an indwelling polyethylene catheter inserted into the jugular vein (16). Each injection of 150 cc. was given in two three-hour infusion periods with an hour rest period intervening.

Source, Chemical and Physical Properties of Parenteral OPG Solution.—*A. Source:* The OPG material supplied consisted of gelatin modified by treatment with a bifunctional condensing agent (glyoxal) and an oxidizing agent (hydrogen peroxide) after the method of Campbell, *et al.* (2). Preparations show a considerable degree of heterogeneity with respect to molecular size; ethanol fractionation showed that the molecular weight (Mn) ranged from 10,000 to 45,000 with approximately 50% of the particles of 29,000 (Mn) or above (2). Specifications of the parenteral solutions used in this study are as follows:

B. Components of Parenteral Solutions R7, R8, and RG.

Infusion Solution	OPG, % (W/V)	Casein Hydrolysate, % (W/V)	Thiamin, % (W/V)	Riboflavin, % (W/V)	Nicotinamide, % (W/V)	Glucose (Anhyd.), % (W/V)
R7	0	26	0.03	0.012	0.3	0
R8	6	0	0	0.0096	0.0	0
RG	0	4.3	0.005	0.01	0.05	10

C. Properties of Parenteral, Nutrient, R8.

Supplier Lot Number	OPG, % (W/V)	pH	Per Cent Residual H_2O_2	Viscosity to Water 6% SN.	Relative (37°C.) 5% Sol.
V64 P139	6	5.18	...	3.46	2.92
V168 P17	5.4	5.41	...	...	2.05
S219XA	6	4.92	nil	...	...

* Received March 11, 1953, from the Division of Pharmacology and Toxicology, Atomic Energy Project, School of Medicine, University of California at Los Angeles.

This article is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

We wish to thank Dr. Alvin E. Lewis and Mrs. Dorothy N. Highby for the preparation and interpretation of the histological material and to express our appreciation to Don Baxter, Inc., of Glendale, California, for their cooperation in the preparation of the parenteral solutions.

D. Elementary Composition of R8 as derived from Spectrographic Analysis of S23 OX.

Gm./100 Ml.		70 Or More P. P. M.		50 Or Less P. P. M.	
Ash	0.1	Aluminum	70	Bismuth	10
Sodium — not determined		Iron	80	Copper	50
Calcium	0.002	Manganese	200		
Magnesium	0.001				
Potassium	0.005				
Silicon	0.001				

Prior to injection 25 ml. of R7 solution was mixed with 125 ml. of R8 solution at room temperature. The RG solution was used as supplied. Animal groups *C* and *D* (Fig. 1) received intravenous infusions of the R7 and R8 mixture while animal group *E* (Fig. 1) received the RG solution.

In the feeding phase of the experiments, the animals received the regular colony diet of Albers Green Rabbit Pellets, high protein pellets (Albers Calf Manna) and greens.

OPG animals sacrificed at the end of the injection (sacr. twelfth day—12D-OPG) or recovery (sacr. twenty-fifth day—25D-OPG) periods, those removed from the experiment or those that died during the experimental periods were autopsied and tissue specimens of lungs, liver, kidney, spleen, pancreas, adrenal, and muscle routinely stained with hematoxylin-eosin and examined histologically. Liver and kidney specimens were also fixed in absolute alcohol and methyl salicylate and studied for glycogen content with Best's Carmine stain.

Body weights were taken daily and correlated with the appearance and the activity of the animals, deviations of which would indicate the onset of toxicity (16).

RESULTS

Figure 1 shows the effect of intravenous OPG infusions upon the body weight of animals that were fed (group *C*) or fasted (group *D*) as compared with control groups (*A*, *B*, and *E*).

By the end of the injection period, at which control groups *A*, *B*, and *E* and approximately one-half of groups *C* and *D* were sacrificed, Group *A* gained 7%; *B* lost 28%; *C* gained 2%; *D* lost 14%, and *E* lost 14% of their initial body weight respectively. Body weight changes of group *C* were characterized by two peaks, one of +3% on the tenth day of infusion and one of +22% on the twenty-first through the twenth-fifth day of the recovery period.

OPG infused animals (groups *C* and *D*) were ill at the end of the eleven-day injection period as shown by their unkempt appearance, listlessness, and in-

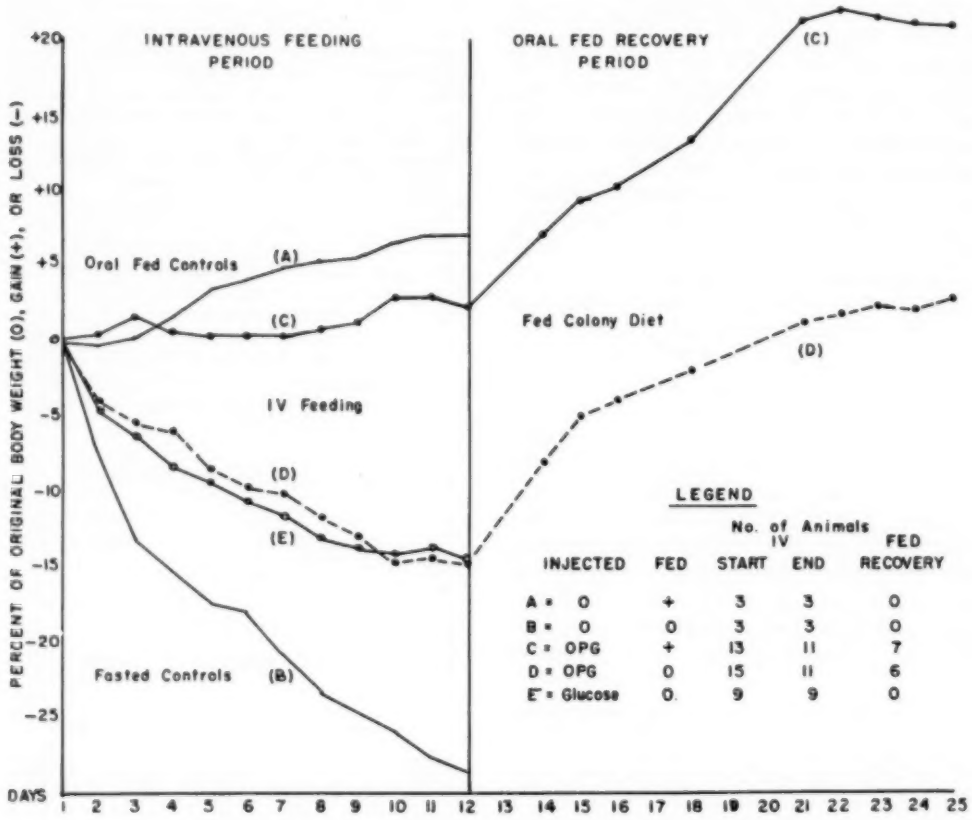


Fig. 1.—Effect of intravenous feeding and recovery on body weight.

activity, as contrasted with the active, healthy, normal appearance of groups *A* and *E* and the generally weak condition of the fasted controls (group *B*).

Two animals died during the experiment (group *D*, 8- and 25-D). Four animals exteriorized their indwelling catheters, were sacrificed, autopsied, and specimens taken for histological study following 4, 7, 9, and 10 injections. Autopsy and histological data for the animal groups are shown in Table I.

DISCUSSION

The present investigation was designed to test and compare the effects of repeated intravenous injections of OPG solutions with results previously obtained employing tapioca dextrin, similarly administered (16). OPG infused rabbits developed symptoms of chronic toxicity and tissue changes that were irreversible, whereas tapioca dextrin solutions were well tolerated by the majority of animals and the tissue changes were reversible.

TABLE I.—AUTOPSY FINDINGS AND HISTOLOGICAL CHANGES IN RABBITS RECEIVING OPG

Animal Groups			Day Sacr.		Increased Fluid[a]		Liver	Kidney	
	Injected	Fed	12	25	Peritoneal	Pleural	Glycogen[b]	Degeneration[c]	Color[d]
A	0	+	+		0	0	4+	0	N
B	0	0	+		0	0	0	0	L. Br.
C	OPG	+	+		+(5 of 6)	+(2 of 6)	4+	4+	Y. Br.
				+	0	0	3+ to 4+	3+ to 4+	Y. A.
D	OPG	0	+		2+(4 of 6)	0	2+	2+	Y. Br.
				+	±(2 of 6)	0	2+ to 3+	2+ to 3+	Y. A.
E	Glucose	0	+		±(2 of 9)	0	4+	0	Pale

[a] Increased fluid; + = 15 to 25 cc.; ± = 5 to 10 cc.
[b] Normal glycogen content = 4+; approximately 50% = 2+.
[c] Uniform hydropic degeneration in convoluted tubules = 4+; greatly reduced yet present in 50% of the tubules 2+.
[d] Light brown (L. Br.); yellowish-brown (Y. Br.); transparent yellow-amber (Y. A.); normal (N).

At autopsy the internal organs of the control groups *A*, *B* and *E* appeared essentially normal. Increased pleural and peritoneal fluid occurred in 2 animals that died and in 2 of 6 of group *C* (12D-OPG). Increased peritoneal fluid only was found in the majority of 12D-OPG animals; in 2 of 9 in group *E* (12-D glucose); in 2 of 6 in Group *D* (25D-OPG) and in 4 animals with exteriorized catheters. The livers of 12-D OPG animals were soft in consistency, wet, and protruding on the cut surface, although the edges were sharp and varied from a normal to a light brown color, as contrasted with a firm consistency, a dry granular cut surface and a light brown to normal color of 25D-OPG animals. The kidneys of 12D-OPG animals were large, soft in consistency, glossy on the cut surface, and varied in color from a light to a yellowish-brown, whereas in 25D-OPG animals the kidneys were smaller, firm in consistency, and varied from a yellow brown to a clear translucent amber color. Grossly, the spleens of 12D-OPG groups were large, firm, with rounded edges, and normal color, while those of the 25D-OPG animals were essentially normal. Muscle, adrenal, lung, and pancreas appeared essentially normal in animals sacrificed at both periods.

Histologically, muscle, adrenal, spleen, and pancreas were also essentially normal. Small discrete emboli were observed in the peripheral vessels of the lungs but the endothelium of the vessel walls was not involved. Foam cells were not seen in those parts of the reticuloendothelial system examined. Liver slides of animals (sacr. twelfth and twenty-fifth days) showed a normal architecture, individual cells were large, pale, contained coarse granules, small vacuoles, and were glycogen-laden. Variations in liver glycogen content, as determined histologically, have been listed in Table I. Kidney sections of 12D-OPG animals showed general swelling and pallor of all tubular epithelium, the cytoplasm was clear or composed of large confluent vacuoles, the nuclei were intact, and the glomeruli were normal. Hydropic degeneration signifies histological kidney changes and the extent to which these were irreversible is shown in Table I.

Both solutions were injected into animals having a normal circulating blood volume, a factor that would tend toward increased elimination, as shown by Price and Longmire (17) and therefore favor tolerance rather than increase sensitivity. Animals previously subjected to hemorrhage retain plasma substitutes longer than normal animals (17). Mice following shock or burns have a reduced tolerance for plasma substitutes, Millican, *et al.* (5). Detrick, *et al.* (18), reported an increased sensitivity of irradiated rabbits to tapioca dextrin infusions while Allen, *et al.* (19), observed that even whole blood was detrimental to irradiated dogs.

The chronic toxic effects that followed OPG infusions cannot be attributed to the infusion volume alone because the tapioca dextrin animals similarly injected developed no ill effects in the majority of cases. Daily dosage volumes were large, being equivalent to 7.7% of the body weight, approximately five times the dosage used clinically, and were equal to or greater than the complete daily blood volume replacement in the animal for eleven consecutive days. Millican, *et al.* (5), observed toxicity in normal mice given OPG infusions equal to 5–15% of the body weight.

Thorsen and Hint (20) studied the physical-chemical characteristics of intravenously injected colloids. They observed that the use of native macromolecular particles resulted in death of infused animals whereas hydrolyzed colloids (reduction of molecular size) were tolerated. Furthermore, they found colloids have a specific concentration and an average molecular weight necessary for aggregation and sedimentation of red blood cells which increases with molecular weight and decreases with the amount of low molecular weight colloid present. OPG is usually injected as a 5% solution; Vars, *et al.* (21), used both 5 and 6% solutions; we employed a 5% solution. A Spinco analytical ultracentrifuge examination showed the OPG used to a polydisperse system, as has been reported by Lippman, *et al.* (4), and the tapioca dextrin previously used was highly polydis-

persed (16). Viscosity determinations showed the latter material to be well below 30,000 molecular weight and the OPG was considered to have a majority of particles above 30,000 mol. wt. since it was produced by the method of Campbell, *et al.* (2), and was the same as that used clinically. OPG production batches did vary somewhat because one jelled and another did not when stored in the refrigerator at 8°. The type and dimensions of the tapioca dextrin molecule were not determined and with respect to the OPG molecules only speculations have been made (2). Of the two macromolecular materials tested, the greatest tolerance and reversibility of tissue damage occurred with the use of tapioca dextrin, a polysaccharide of low molecular weight, highly polydisperse, and given in the highest concentration. Although not a perfect correlation from this work it would appear that the development of toxicity and the duration of tissue damage are dependent on characteristics of the material administered other than its content of small particle sizes as postulated by Lippman, *et al.* (4).

Since the body weight values for Group *D*, Fig. 1, are well above those of the starved control group *B* and are approximately equal to the weight values for the glucose infused group *E*, some of the injected OPG material may have been metabolized as shown by the studies of Gray, *et al.* (14, 15). A similar weight change was observed following tapioca dextrin infusions (16). Hydropic degeneration was seen in the convoluted tubule epithelium of the kidney following tapioca dextrin and OPG infusions, as well as following the intravenous injection of other plasma substitutes (6). The swelling of the tubule cells is usually considered, in part, as a manifestation of the retention of the macromolecular material injected (6). It appears that the small tapioca dextrin particles in the tubule cells were either metabolized or thrown out as shown by the reversibility of the kidney damage whereas the retained OPG particles were not. Such lack of information regarding the ultimate fate of retained colloids has restricted their acceptance for clinical use (22).

Histologically the hydropic degeneration in the kidney was the most outstanding tissue change observed. It appeared following 4, 7, 9, and 11 consecutive daily OPG injections. The convoluted tubules and Henle's loops, but not the glomeruli were involved. Oliver (3) determined the distribution of OPG damage in the nephron. Our kidney slides compared favorably with illustrations of Hartman (6). Foam cells were not observed in the reticuloendothelial system but were a characteristic finding of others following plasma substitute administration (6). Endothelial degeneration of the blood vessels, as reported by Hartman (6) following the administration of polyvinylpyrrolidone was lacking, but small discrete emboli were noted in peripheral vessels of the lung. The origin of the emboli was not determined but caseous material about the indwelling cannula or pseudoagglutination (21) was considered a likely source. The liver cells appeared essentially normal for animals. An occasional giant cell of the Langham's type heavily loaded with lymphocytes was observed, but foam cells were lacking.

CONCLUSIONS

1. Eleven consecutive daily intravenous injections containing 5 per cent oxypolygelatin in a nutrient solution, each equivalent to 7.7 per cent of the body weight, produced physical deterioration and illness in rabbits and a comparatively low mortality ratio.

2. Hydropic degeneration in the convoluted tubule epithelium occurred following 4 and 7 injections and was present in all OPG infused animals sacrificed at the end of the eleven-day injection period, but the lesions were not reversible fourteen days after the injections were stopped. The glomeruli were not involved. Foam cells were not present in the reticuloendothelial system. Small discrete emboli were frequently seen in the peripheral vessels of the lung but the endothelium of the blood vessels was normal.

3. From comparative experimental results it would appear that physical deterioration, illness, and the irreversible hydropic degeneration of the tubule epithelium were dependent upon characteristics of the injected material other than the presence of or proportion of small molecular particles in the polydisperse OPG system.

REFERENCES

(1) Pauling, L., Campbell, D. H., Abrahamsen, N., Feigen, G., Lanni, F., Le Rosen, A. L., and Swingle, S., *Bull. Blood Substitutes*, N.R.C. C.M.R., Appendix P, P1123 (1944).
(2) Campbell, D. H., Koepfli, J. B., Pauling, L., Abrahamsen, N., Danliker, W., Feigen, G., Lanni, F., and Le Rosen, A. L., *Texas Repts. Biol. & Med.*, **9,** 235(1951).
(3) Oliver, J., *Blood Substitutes*, C.R.M., OSRD. OEMcmr. 330 M-2645(1943).
(4) Lippman, R. W., Marti, H. U., Oliver, J., *Federation Proc.*, **11,** 96(1952).
(5) Millican, R. C., Rosenthal, S. M., Mowry, R. W., and Stohlman, E. F., *ibid.*, **10,** 325(1951).
(6) Hartman, F. W., *Arch. Surg.*, **63,** 728(1951).
(7) Hamilton, A. S., and Parkins, W. M., *Am. J. Med. Sci.*, **201,** 808(1945).
(8) Gropper, A. L., Cockrell, E. W., Raisz, L. G., and Pulaski, E. J., *Am. J. Physiol.*, **169,** 749(1952).
(9) Hamilton, A. S., Parkins, W. M., and Waltzer, F., *ibid.*, **150,** 641(1947).
(10) Govier, W. M., and Colovos, G. C., *Ann. N. Y. Acad. Sci.*, **55,** 491(1952).
(11) McCarthy, M. D., and Parkins, W. M., *Am. J. Physiol.*, **150,** 428(1947).
(12) McCarthy, M. D., and Draheim, J. W., *Proc. Soc. Exptl. Biol. Med.*, **79,** 346(1952).
(13) Raisz, L. G., *Federation Proc.*, **11,** 126(1952).
(14) Gray, I., Süüteri, P. K., and Pulaski, E. J., *Proc. Soc. Exptl. Biol. Med.*, **77,** 626(1951).
(15) Gray, I., and Pulaski, E. J., *ibid.*, **78,** 421(1951).
(16) Detrick, L. E., Rhodes, B., and Haley, T. J., THIS JOURNAL, **41,** 614(1952).
(17) Price, P. B., and Longmire, W. P., *Bull. Johns Hopkins Hosp.*, **71,** 51(1942).
(18) Detrick, L. E., Lewis, A. E., Haley, T. J., and Rhodes, B., Report UCLA-181, Atomic Energy Project, University of California at Los Angeles, January, 1952.
(19) Allen, J. G., Basinger, C. E., Landy, J. J., Sanderson, M. H., and Enerson, D. M., *Science*, **115,** 523(1952).
(20) Thorsen, G., and Hint, H., *Acta Chir. Scand. Suppl.*, **154,** 21(1950).
(21) Vars, H. M., Parkins, W. M., and Perlmutt, J. H., *Ann. N. Y. Acad. Sci.*, **55,** 496(1952).
(22) Stormont, R. T., *J. Am. Med. Assoc.*, **147,** 658 (1951).

Separation of Some of the Opium Alkaloids by Surface Chromatography*

By MITCHELL L. BORKE† and ERNST R. KIRCH

Some of the opium alkaloids and meconic acid were separated and identified by the use of surface chromatography. The strips were coated with a fluorescent adsorbent buffered at *p*H 6.6. The chromatograms were developed using 1,4-dioxane. Identification of the individual opium alkaloids on the strips was accomplished by means of R_f values determined with pure samples, coupled with the observation of the extinction of fluorescence at the area of adsorption of the alkaloids. As little as 15–20 micrograms of papaverine hydrochloride, narcotine, or meconic acid, and 50 micrograms of morphine, codeine, or narceine could be detected by this method.

THE IDENTIFICATION reactions for the detection of opium alkaloids available at present do not permit one to establish the presence of a particular alkaloid in the crude drug unless the alkaloid is separated from it by a rather tedious extraction process. The coloring matter present in the crude drug obscured at times the qualitative color tests so commonly used to identify the alkaloids.

Chromatographic methods for the isolation of opium alkaloids have been used primarily for the purpose of purifying the alkaloids as one of the steps in their quantitative determinations (1). Reports pertaining to the qualitative chromatographic separation have been published by Levi and Castelli (2) and Kocsis and Hollo (3). Munier and Macheboeuf (4) approached the problem of separating morphine, codeine, and thebaine by means of paper chromatography. The R_f values[1] reported were 0.68 for morphine and 0.73 for codeine. This difference of 0.05 can hardly be considered significant because the R_f values of substances chromatographed under identical conditions frequently exhibit a degree of variability even higher than 5 per cent.

Searching for a chromatographic method which would permit us to achieve a better separation of morphine, codeine, and some other constituents of opium, we turned to surface chromatography introduced by Meinhard and Hall (5) and by Kirchner, Miller, and Keller (6). We felt that particularly the technique employing a fluorescent adsorbent as described by Kirchner, *et al.*, might be applicable to the described analytical procedure because no qualitative color tests for the detection of opium alkaloids were found to be sensitive enough to permit the locating of their spots on the developed chromatostrips.

EXPERIMENTAL

The chromatographic chamber used in these determinations consisted of a glass jar 45 cm. high and 14 cm. in diameter. The solvent was contained in a Petri dish placed at the bottom of the jar. The jar was made airtight using a glass plate sealed by starch paste prepared according to Burton, Zaffaroni, and Keutmann (7). The glass strips, 2.5 cm. x 43 cm., coated with the adsorbent, were placed in an upright position with their lower ends immersed in the solvent, and thus developed by the ascending technique.

The adsorbent paste was composed of 10 Gm. of silicic acid (Mallinckrodt, Analytical Reagent), 10 Gm. of heavy magnesium oxide (Mallinckrodt, U. S. P. XIII), 4 Gm. of plaster of Paris, 0.250 Gm. of phosphorescent zinc silicate No. 609 (E. I. du Pont de Nemours & Co.) and 0.250 Gm. of phosphorescent zinc cadmium sulfide No. 1502 (E. I. du Pont de Nemours & Co.). The above quantities were thoroughly mixed in a mortar just prior to each run. Then 38 ml. of the phosphate buffer (*p*H 6.6) was added to the mixture and stirred for two minutes until a creamy suspension was obtained. The mixture was spread on the glass strips so that at least three-quarters of the strip were covered with the adsorbent about 1 mm. in thickness. The strips were tapped slightly to insure an even distribution of the adsorbent, and left for a few minutes to permit settling and partial drying of the coating. The partially dried strips were transferred to an oven and dried at 80–90°.

Spotting of stock solutions onto the strips was accomplished by the use of calibrated capillary pipettes from which a measured amount of stock solution was allowed to flow onto the strip. The spots on the strips were then allowed to dry by evaporation of the solvent used for the stock solutions.

About one to two hours prior to each run the 1,4-dioxane was placed in the Petri dish in the chamber, and the latter sealed. This was done to saturate the atmosphere within the jar with the developing solvent. It is imperative to have the atmosphere inside the chamber saturated with the 1,4-dioxane in order to prevent any evaporation from the strips of said solvent during the developing of the chromatograms.

Of the number of solvents investigated which included methanol, ethanol, ethyl acetate, cyclohexa-

* Received February 24, 1953, from the Department of Chemistry, College of Pharmacy, University of Illinois, Chicago 12, Ill.

† Abstracted in part from a thesis presented to the Graduate College of the University of Illinois by Mitchell L. Borke, in partial fulfillment of the requirements for the degree of Master of Science.

[1] R_f value is the ratio of the distance traveled by the band to the distance traveled by the solvent, measured from the point at which the substances under test were applied to the strip.

nol, cyclohexanone, and others, the most efficient separations were obtained with 1,4-dioxane.

Following the development of the chromatostrips, the identification of the separated alkaloids was accomplished by exposing the strips to ultraviolet light, marking the areas of highest alkaloidal concentration with a sharp pencil, and calculating the R_f values. Results obtained by chromatographing five opium alkaloids and meconic acid on separate strips but in the same chamber; i. e., under the same set of conditions, as shown in Table I, represent the average of four or more determinations.

TABLE I.—RF VALUES OF FIVE OPIUM ALKALOIDS AND MECONIC ACID

	R_f
Morphine	0.39
Codeine	0.62
Papaverine HCl	0.88
Narcotine	0.92
Narceine	0.00
Meconic acid	0.00

When the stock solutions of the above-mentioned five components of opium were pooled to give solutions containing equal concentrations of each, and when these mixtures were chromatographed, the R_f values of the separated substances shown in Table II checked fairly well with those presented in Table I.

TABLE II.—RF VALUES OF FIVE OPIUM ALKALOIDS AND MECONIC ACID OBTAINED BY CHROMATOGRAPHING THEIR MIXTURES

	Mixture I	Mixture II
Morphine	0.40	0.38
Codeine	0.57	0.58
Papaverine HCl	0.89	0.88
Narcotine	0.95	0.92
Narceine and meconic acid	0.00	0.00

No appreciable differences in the R_f values were observed when heavy magnesium oxide manufactured by two other companies was used in the determinations.

DISCUSSION

The most efficient separation was achieved when a phosphate buffer at *p*H 6.6 was used in the preparation of the adsorbent mixture and 1,4-dioxane was the developing solvent.

When the buffer was added to the developer only, or both to the developer and to the adsorbent mixture, the spots of morphine and codeine were ill defined and spread over the entire width of the strip. Furthermore, under both sets of conditions, papaverine hydrochloride and narcotine moved with the solvent front.

Buffers below *p*H 6.6 could not be tested because zinc silicate and zinc cadmium sulfide lost their fluorescent properties below *p*H 6.6. It should be noted that even under the optimum conditions of the experiment narceine and meconic acid did not move (Table I). The method served as a suitable one for the separations of these from the other alkaloids, but was unsuitable for the separation of narceine from meconic acid.

The sensitivity of the separations varied with the substances under study. At least 50 μg. of morphine, codeine, or narceine had to be applied to the strip in order to permit the locating of their spots. Papaverine hydrochloride, narcotine, and meconic acid could be located when 15–20 μg. were applied to the starting line. Although the R_f values of papaverine hydrochloride and narcotine were very close, their spots could be differentiated by a violet and a blue color, respectively. Meconic acid was identified by a dark violet spot at the point of application. When the acid was omitted from the mixture, narceine was represented by a light blue spot on the starting line.

The temperature at which the determinations were carried out stayed within a five degree range (28–33°) during the entire period of the investigation. The variations in the R_f values for morphine and codeine within this temperature range did not exceed 0.02.

In an attempt to test the efficiency of this method of analysis, determinations were carried out on the following: (*a*) an opium extract prepared by extracting of opium, U. S. P. XI, (Merck & Co.) according to the first step in the official assay of opium; (*b*) morphine prepared according to the directions for the assay of opium in U. S. P. XIV; (*c*) Elixir Terpin Hydrate with Codeine, N. F.; and (*d*) a methanol extract of opium prepared by the procedure of Klee (8).

The R_f values of the separated constituents of opium extract are presented in Table III.

TABLE III.—*R*F VALUES OF THE SEPARATED OPIUM EXTRACT CONSTITUENTS

	R_f
Morphine	0.38
Codeine	0.51
Papaverine HCl	0.86
Narcotine	0.90
Narceine and meconic acid	0.00

These R_f values check fairly well with those of the simpler mixtures presented in Tables I and II.

Only morphine (R_f values 0.37 and 0.40) was located on the developed strip when this alkaloid prepared as mentioned in (*b*) was chromatographed.

On the strips containing Elixir Terpin Hydrate with Codeine, the alkaloid was observed as a violet spot (R_f values 0.56 and 0.59). The chromatostrips with the samples of the methanolic opium extract prepared for the colorimetric determination of morphine by the method of Klee (8) a papaverine spot (R_f 0.89 and 0.91) in addition to morphine (R_f 0.39 and 0.41).

SUMMARY

A method has been described for the separation of the major opium alkaloids and meconic acid by surface chromatography using a buffered fluorescent adsorbent and 1,4-dioxane as the solvent. The separated compounds were then identified as violet or blue spots on a light green

background. Some experiments were carried out to evaluate the practical applications of this method.

REFERENCES

(1) Stolman, A., and Stuart, C. P., *Analyst*, **74**, 536 (1949).
(2) Levi, J. R., and Castelli, F., *Gazz. chim. ital.*, **68**, 459 (1938).
(3) Kocsis, E. A., and Hollo, Z., *Z. anal. Chem.*, **124**, 35 (1942).
(4) Munier, R., and Macheboeuf, M., *Compt. rend.*, **230**, 1177(1950).
(5) Meinhard, J. E., and Hall, N. F., *Anal. Chem.*, **21**, 185(1949).
(6) Kirchner, J. G., Miller, J. M., and Keller, G. J., *ibid.*, **23**, 420(1951).
(7) Burton, R. B., Zaffaroni, A., and Keutmann, E. H., *J. Biol. Chem.*, **188**, 763(1951).
(8) Klee, F. C., M. S. Thesis, University of Illinois, 1952.

The Use of Chelating Agents for Accelerating Excretion of Radioelements*

By HARRY FOREMAN†

It has been demonstrated that certain chelating agents can be used to hasten the excretion of radioelements which have become deposited in the body. Specifically, the calcium chelate of ethylenediamine tetracetic acid has been shown to accelerate the urinary excretion of yttrium and plutonium. Similarly, Fe-3, a commercial chelating agent was found to accelerate the urinary excretion of plutonium. The results were produced whether the chelating agent was given shortly after or early after the radioelement was administered. The increased excretion of the plutonium was reflected in a lower Pu content in the tissues of the treated animals.

IN THE PAST, attempts to remove radioactive elements from the body have included: low calcium diets, parathormone, viosterol, ammonium chloride, calcium gluconate (1, 2), low phosphorus diets (3), and zirconium citrate (4). Of these, only zirconium citrate was shown to have an appreciable measure of effectiveness. The present study reports another approach to the problem of accelerating the excretion of radioelements, namely, the use of chelating agents.

Of many chelating agents considered, ethylenediamine tetracetic acid (EDTA) was chosen for study. This agent was selected because it forms stable compounds with many metal ions and hence has a strong tendency to remove such ions from insoluble combinations, i. e., it will dissolve such salts as calcium oxalate, barium sulfate, and lead phosphate in neutral and alkaline solutions (5). Moreover, it has suitable characteristics for *in vivo* application. It forms serum-soluble chelates which are not readily broken down in the body but are rapidly eliminated by the kidney. When administered as a neutral Na salt, EDTA combines avidly with serum calcium and produces death in hypocalcemia with relatively small doses, i. e., approximately two hundred mg./Kg. of body weight in rats. However, when administered combined with an equivalent weight calcium ion, a negative calcium balance is prevented and the compound is rendered relatively nontoxic. A dose equivalent to 3 Gm./Kg. of body weight, injected intraperitoneally into rats, is required to produce death in approximately fifty per cent of the injected animals. Under these conditions EDTA will still chelate a large number of metals, namely the metals which displace the calcium from combination with the EDTA because they form more stable chelates.

METHODS

In the experiments to be described, yttrium-91 and plutonium 239 were used. Both of these elements are of interest as potentially serious health hazards in that they are bone seekers with long biological half-lives. Female Curtis-Dunning rats weighing approximately 250 Gm. were employed. The yttrium experiment was performed using 4 groups of 3 rats each. Each rat was injected intravenously with approximately 25 μc. of carrier-free Y^{91} in 0.5 cc. of solution of yttrium chloride in isotonic saline freshly adjusted to *p*H 6. The various groups received calcium EDTA by different

* Received March 25, 1953, from the Crocker Laboratory, Radiation Laboratory, University of California, Berkeley, California.
This document is based in part on work performed under Contract 7405-eng-48-A for the Atomic Energy Commission.
† Atomic Energy Commission Postdoctorate Fellow in the Division of Medical Sciences of the National Research Council. Present Address: University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico.
The writer wishes to express his gratitude for the technical services of the staff of the Crocker Laboratory. The advice and generous cooperation of Dr. Joseph G. Hamilton, Dr. Arthur E. Martell, Dr. Warren M. Garrison, Mr. H. Ralph Haymond, and Dr. Frederick C. Bersworth are greatly appreciated.
The chelating agents were generously supplied through the courtesy of the Bersworth Chemical Co., Framingham, Massachusetts.

TABLE I.—ADMINISTRATION OF Ca EDTA TO RATS INJECTED WITH Y^{91}

Time after Y^{91} Administration	A-B	C	D
2 hours	20 Mg. i. v.	none	none
48 hours	none	20 Mg. i. m.	20 Mg. s. t.
20th through 23rd day	25 Mg. i. p.	25 Mg. i. m.	25 Mg. s. t.
48th through 52nd day	25 Mg. i. p., twice daily	25 Mg. i. m., twice daily	25 Mg. s. t., twice daily

i. v. = Intravenous; i. m. = intramuscular; s. t. = stomach tube; i. p. = intraperitoneal.

routes, intraperitoneally, intramuscularly, and by stomach tube under a time dosage schedule as indicated in Table I.

Urine and feces were collected separately, daily for the first three days and then pooled at three-day intervals. The yttrium content was determined according to the method described by Scott, *et al.* (6). The results were expressed on the basis of per cent of the originally administered dose per gram of dry ash.

For the plutonium study, a dose of 15 μg. of Pu^{239} as sodium plutonyl acetate in 0.5 cc. of isotonic neutral solution containing 15 mg. of citric acid was injected into each of 12 animals. Six of the animals were set aside as controls and received no treatment. The other group received different chelating agents at various time periods as indicated in Table II. The feces and urine were collected separately each day for a sixty-two-day period. On the sixty-second day the animals were sacrificed and tissues taken for assay. Both the excreta and tissue were dry-ashed, processed, and counted according to the method of Scott, *et al.* (7).

TABLE II.—ADMINISTRATION OF CHELATING AGENTS TO RATS INJECTED WITH Pu^{239}

Time after Injection of Radioelement	Amount and Type of Chelating Agent
18 hours	25 Mg. Ca EDTA, i. p.
13th through 17th day	50 Mg. Ca EDTA, i. p., twice daily
35th through 38th day	100 Mg. Ca Citrate, i. p.
48th through 52nd day	100 Mg. Fe-3[a], i. p.

[a] $HO{-}CH_2{-}CH_2$ \
$\quad\quad\quad

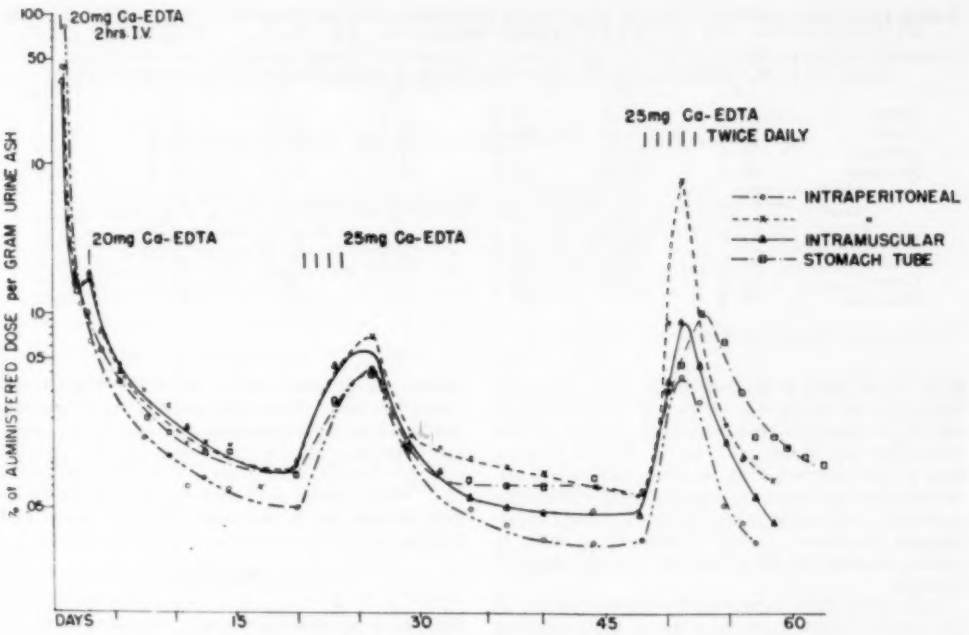


Fig. 1.—The effect of chelating agents on urinary excretion of Y^{91}.

It is apparent from a consideration of the above data that for effective therapy involving long-lived elements, prolonged continued treatment will be necessary even when as profound an alteration as a tenfold increase in the urinary excretion rate is effected. In the rat, at the time when plutonium is well fixed in the body, the urinary-fecal ratio is approximately one to ten. Hence a tenfold increase in urinary excretion will not affect the over-all excretion of the radioelement very markedly. In the human being the situation could be more favorable. The urinary-fecal ratio when the plutonium is well

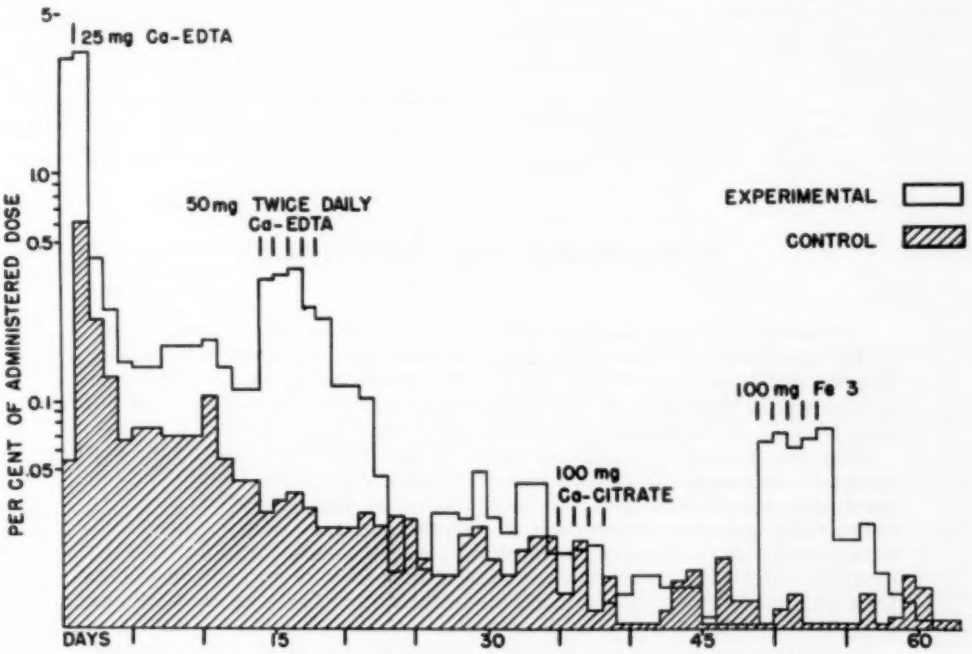


Fig. 2.—The effect of chelating agents on urinary excretion of PU^{239}.

TABLE III.—DISTRIBUTION STUDIES FOLLOWING ADMINISTRATION OF CHELATING AGENTS TO RATS INJECTED WITH Pu^{239}

	Control Group						
	A	*B*	*C*	*D*	*E*	*F*	Average
Urine	3.7	3.3	3.2	2.8	3.7	3.1	3.3
Feces	40.5	35.8	34.8	30.4	40.5	34.1	36.1
Carcass	14.5	10.0	7.6	7.4	11.1	12.6	10.7
Skeleton	40.5	51.2	54.5	59.4	44.4	50.1	50.0
	Experimental Group						
	A	*B*	*C*	*D*	*E*	*F*	Average
Urine	14.5	11.3	13.2	13.8	14.0	14.1	13.0
Feces	49.0	38.1	44.3	46.6	47.1	47.5	45.4
Carcass	7.0	12.3	6.0	6.1	5.5	7.9	7.5
Skeleton	29.4	38.1	36.5	33.4	33.4	30.2	33.5

Figures represent per cent of dose recovered.

fixed in the body is approximately two to one and here a tenfold increase in urinary excretion may result in an approximately sevenfold increase in the total excretion of the plutonium. In this connection it is of interest that repeated day-to-day administration of chelating agents in this experiment continued to bring forth high levels of urinary radioelement excretion. It still remains to be seen, of course, whether this effect could be brought about in humans.

Aside from their merits as therapeutic agents in this type of application, it is likely that chelating agents could prove to be of value as a diagnostic aid. By increasing urinary excretion of such radioelements as Pu, they could raise the threshold of detection of the elements in the urine and thereby facilitate greatly the estimation of the body burden of the element.

It is significant that the postulation from *in vitro* data that plutonium and yttrium would displace the calcium from combination with the EDTA was verified *in vivo*. One might predict that strontium would not be mobilized from the body by this treatment because of the fact that calcium forms a stronger chelate with EDTA than does strontium (6). From similar considerations, one would not expect radium to be removed from the body with EDTA.

REFERENCES

(1) Aub, J. C., Evans, R. D., Gallagher, D. M., and Tibbetts, D. M., *Ann. Internal Med.*, **11**, 1443(1938).
(2) Craven, L. F., and Schlundt, H., *J. Am. Med. Assoc.*, **105**, 959(1935).
(3) Copp, H. D., Axelrod, D. J., and Hamilton, J. G., *J. Roent. Rad. Ther.*, **58**, 10(1947).
(4) Shubert, J., *J. Lab. Clin. Med.*, **34**, 313(1949).
(5) *The Versenes*, Tech. Bulletin No. 2, Section L, P. 3, Bersworth Chemical Company, Framingham, Mass. (1950).
(6) Scott, K. G., Crowley, J. F., Fishler, M. E., Enteman, C., Chaikoff, I. L., Loomis, F., and Barber, A. J., *Manhattan District Declassified Report MDDC 1275(1948)*.
(7) Scott, K. G., Axelrod, D. J., Fisher, H., Crowley, J. F., and Hamilton, J. G., *J. Biol. Chem.*, **176**, 283(1948).

Symposium on Antibiotics

A SYMPOSIUM ON ANTIBIOTICS will be held on October 28, 29, and 30, 1953, in the auditorium of the Department of Health, Education, and Welfare, Fourth and Independence Avenue, S. W., Washington, D. C. This symposium will be sponsored by the Department of Health, Education, and Welfare, Division of Antibiotics of the Food and Drug Administration.

Since the symposium is planned primarily as a means of reporting the most recent developments in the field of antibiotics, contributors will offer only previously unpublished data. It is planned that the symposium cover the entire field, including information on the emergence of resistant organisms, development of sensitivity (particularly anaphylactoid reactions), blood dyscrasias and other toxic reactions, clinical usefulness of new antibiotics and their pharmacology, problems involving antibiotics in feeds, research and development problems, and possible new uses of antibiotics.

In addition to contributors of papers, it is planned to invite a few outstanding clinicians in the field to speak on key subjects.

Anesthesia. XLIII. The Estimation of Anesthetic Trifluoroethyl Vinyl Ether in Aqueous Solution*

By FREDERICK K. BELL and JOHN C. KRANTZ, Jr.

The instantaneous reaction between trifluoroethyl vinyl ether and bromine water, resulting in a decolorization of the latter, has been adapted for the colorimetric estimation of this ether in aqueous solution. Under suitable conditions the degree of decolorization is proportional to the amount of the ether used. The method is simple and rapid and may be applicable to the examination of numerous other substances which are highly reactive toward bromine water in a similar manner.

In the course of the study of new inhalation anesthetic agents in this laboratory, it has been frequently desirable to determine the oil/water distribution since this factor is regarded by some as of significance in the mechanism of anesthetic action. In most cases a precise determination is not required, and therefore there is considerable latitude in developing a suitable method. A case in point concerns recent studies on trifluoroethyl vinyl ether (1). It is the purpose of this communication to describe a method which has been devised for the estimation of this ether in aqueous solution.

The procedure is empirical and is based on the observation that under certain conditions a bromine solution is decolorized by an aqueous solution of trifluoroethyl vinyl ether, and that the degree of decolorization is proportional to the amount of ether which, therefore, can be determined colorimetrically.

The specimen of trifluorethyl vinyl ether used in this study was generously supplied by the Ohio Chemical & Surgical Equipment Company of New York. This material boiled at 42.6° at 760 mm. Hg, and had a density of 1.13 at 25°.

EXPERIMENTAL

Attention was directed first to the estimation of the solubility of trifluoroethyl vinyl ether in water which, obviously, represents a simplified variation of the determination of the ether concentration in the aqueous layer of an oil/water system.

Only one reagent is required for the determination and this is prepared by diluting 55 cc. of saturated aqueous solution of bromine with 500 cc. of water. Six grams of potassium bromide are added and dissolved and the solution is then diluted to 2 L. with water. This solution was stored in a well-stoppered amber glass bottle and for the purposes of these experiments, was found to be stable over a twenty-four hour period. Therefore it was standardized (as indicated below) each day that it was used.

For the colorimetric analysis a Beckman Model DU spectrophotometer was employed. A wave length setting of 385 mμ was selected with a slit width of approximately 0.1 mm. A tungsten lamp in conjunction with a Corning No. 9863 red-purple Corex A filter served as the source of radiation. The absorption cells had a light path of 1 cm. and distilled water was used as the reference standard.

The preparation of a standard ether curve was carried out each day in the following manner. A 50-cc. glass-stoppered volumetric flask containing approximately 40 cc. of water was accurately weighed and then approximately 0.1 cc. of the ether was added. (Preliminary observations indicated that the solubility of the ether in water was in the region of 0.5%.)

The flask was immediately stoppered and shaken for a few seconds. A second accurate weighing was then made. The flask was immediately placed in a mechanical shaker for fifteen minutes, resulting in complete solution of the ether. Toward the end of the shaking period 25-cc. portions of the bromine reagent were pipetted into each of five 50-cc. amber glass-stoppered bottles and promptly stoppered. Immediately after the shaking the ether solution was diluted with water to the mark, yielding a standard aqueous solution of the ether, which was then used at once according to the following protocol.

Bottle No.	Bromine Reagent, Cc.	Standard Ether Solution, Cc.	H_2O, Cc.
1	25	0	8
2	25	2	6
3	25	4	4
4	25	6	2
5	25	8	0

The ether solution was added to each bottle in the indicated amount, using a graduated pipette calibrated to the tip, which was placed beneath the surface of the bromine reagent during the introduction of the ether solution. After each addition the bottle was stoppered and shaken for a few seconds. Distilled water was then added to each flask in the amounts indicated, with subsequent mixing. The absorbancy of each of the five solutions was measured immediately and as rapidly as possible with the spectrophotometer. The entire procedure beginning with the preparation of the standard ether solution did not require more than forty minutes. From the data obtained, a standard ether curve can be prepared by plotting absorbancy against milligrams of ether. Typical daily curves are shown in Fig. 1.

* Received February 13, 1953, from the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore.

This work was conducted with the aid of a grant from the Ohio Chemical & Surgical Equipment Co., New York.

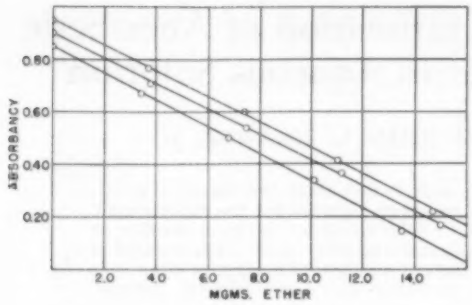


Fig. 1.—Typical standard ether curves.

It will be noted that the procedure is carried out at a constant dilution of 25-cc. aliquots of the bromine reagent with a total of 8 cc. of aqueous medium. All subsequent determinations were carried out on the same basis.

For the determination of the solubility of the ether in water, 1 cc. of the ether was added to 25 cc. of distilled water in a glass-stoppered 50-cc. Erlenmeyer flask. After a fifteen min. period of shaking, the mixture was quickly transferred to a centrifuge tube and centrifuged for two minutes at 2,000 r. p. m. Two-cubic centimeter aliquots of the supernatant layer were pipetted into 25-cc. aliquots of the bromine reagent contained in the amber bottles as indicated above. This was followed by the addition of 6-cc. aliquots of water. The entire procedure was completed within forty minutes. This determination was usually done in triplicate, in which case the average of the three absorbancy values obtained was converted into milligrams of ether from the standard curve, from which the solubility value was readily obtained. A typical series of determinations gave percentage solubility values of 0.39, 0.39, 0.39, 0.38, 0.38, 0.38, 0.39, 0.39, and 0.40, for an average of 0.39 ± 0.01 at room temperature, which varied between 23 and 25°.

For the determination of the oil/water distribution of trifluoroethyl vinyl ether, corn oil, U. S. P., was used. Approximately 25 cc. of the oil was transferred to a tared glass-stoppered 150-cc. Erlenmeyer flask and weighed. Then 2 or 3 cc. of the ether was added, quickly dissolved in the oil, and weighed. These weighings were made to the nearest centigram. Finally, exactly 50 cc. of distilled water was added and the mixture was immediately shaken for fifteen minutes. After this shaking, which should be as vigorous as possible without the formation of a stubborn emulsion, the mixture was allowed to stand for two minutes. A portion of the lower aqueous layer was pipetted off and passed through a Whatman No 2 filter paper. Six centimeter aliquots of this filtrate were used for analysis in the manner previously indicated. A typical group of experiments gave oil/water distribution coefficient values of 97, 94, 93, 90, 85, 91, and 89, for an average value of 91 with a standard deviation of ±4. The room temperature during these experiments varied between 21 and 25°. Blank determinations were carried out on aqueous extracts of the corn oil sample used and revealed that no significant correction was required. The amount of aqueous ether solution of unknown concentration used in all experiments was selected so that the final absorbancy value was in the region of 0.5.

DISCUSSION

The most important factor in the procedure was the observation that the bromine solution used was very photosensitive. Until adequate care was taken to avoid photochemical action the results obtained were very erratic. Therefore in the course of the procedure precautions were taken continuously to minimize the exposure of the bromine solution to daylight.

The values obtained for the solubility of the ether in water show a good agreement under the experimental conditions employed and indicate that complete saturation is rather closely attained during the fifteen minute shaking period. On the other hand, the values of the distribution coefficient show a higher degree of variation and a longer shaking period is clearly indicated. Experiments along this line were not undertaken since it was observed that the aqueous solution of the ether was not stable as evidenced by an appreciable diminution of its reactivity toward bromine after standing thirty to forty minutes. Perhaps the ether is slowly polymerized in aqueous solutions, in which case an accurate solubility determination might involve a complex and difficult procedure.

SUMMARY

1. A colorimetric method for the estimation of trifluoroethyl vinyl ether in aqueous solution has been described. The percentage solubility of the ether in water at room temperature was found to be 0.39 ±1.

2. The method is relatively simple and rapid and may find application in the study of numerous other substances which display a similar reactivity toward bromine water.

REFERENCE

(1) Krantz, John C., Jr., Carr, C. Jelleff, LuGo, and Bell, Frederick K., in Press.

Digitoxin*

By PAUL DEMOEN and PAUL JANSSEN

Digitoxin and related substances are analyzed by chromatographic and spectrophotometric methods. It is shown that the physical and chemical properties of pure digitoxin are still largely unknown.

IN A PREVIOUS article (1) it was shown that there exists a striking lack of suitable purity criteria for digitoxin. The possibilities and limitations of some analytical methods for digitoxin which have been used in this laboratory for a number of years are discussed.

CHROMATOGRAPHY

Stoll (2) described a chromatographic method using silica gel for the separation and determination of gitoxin, and possibly other glycosides, giving a red color with the Keller-Kiliani reagent, in impure digitoxin. We found that this method gives reliable and reproducible results and that it is possible to increase the accuracy by substituting for the weighing method used by Stoll, a colorimetric method for determining the amount of glycoside in each fraction. Stoll demonstrated the presence of a significant amount of gitoxin in U. S. P. Digitoxin Reference Standard. The following example illustrates the possibilities of this modified Stoll method.

Fifty milligrams of digitoxin Eupharma, undried, in 1 cc. of chloroform was eluted through an adsorption column consisting of 36 Gm. of dry silica gel with ethyl acetate saturated with water and containing 0.5% methanol. Forty-eight fractions of 10 cc. each were collected at a rate of 5 cc. per minute. The color of a qualitative Keller-Kiliani test (A. Stoll) with 1 cc. of each fraction was recorded. Fractions 12–23 turned brown (digitoxin and possibly some digitoxigenin glycosides), while fractions 24–30 were colored red (gitoxin and possibly some gitoxigenin glycosides). The quantitative Keller-Kiliani test, as described below, served to determine the amount of glycoside in each fraction. The Baljet reaction was used to check the accuracy of the previous method, and gave identical results. Of the initial amount, 99.3% was found in the fractions 12–30 ($\epsilon \pm 0.3\%$). Fractions 24–30 contained 0.91 mg. ($1.83\% \pm \epsilon 0.6$) of "gitoxin-like" compounds. The sample (1 Gm.) lost 0.66% of its weight by heating for two hours at 105° (U. S. P. XIV).

Analyzing the last "brown" fractions by paper chromatography (3–9), it can be shown that they still contain small amounts of gitoxin-like compounds, which can only be eliminated by repeated chromatography of these brown fractions on silica gel or aluminium oxide (10). When analyzed by these methods all digitoxin samples investigated in this laboratory thus far were found to contain various amounts of impurities.

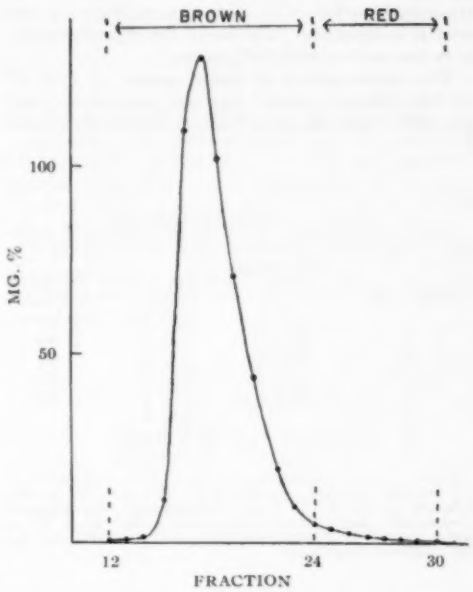


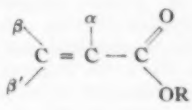
Fig. 1.—Digitoxin chromatography using silicagel.

ULTRAVIOLET SPECTROSCOPY

The quantitative determination of U. V. spectra of digitaloid aglycones and glycosides is difficult owing to the unfavorable position of the absorption maximum (215–222 mμ). The following data are recorded in the literature:

Canbäck (14): $\lambda_{max.}^{EtOH} = 219$ mμ; $\log \epsilon = 4.15$.
McChesney, *et al.* (11): $\lambda_{max.}^{EtOH} = 215$ mμ; E (1%, 1 cm.) = 263, $\log \epsilon = 4.30$.
Kaern (12): $\lambda_{max}^{EtOH\ abs.} = 218$–220 mμ; E (1%, 1 cm.) = 213; $\log \epsilon = 4.21$.

Molecules containing the conjugated system



all show a maximum of absorption between 207 and 230 mμ ($\log \epsilon = 4.0 \pm 1.0$). The position of $\lambda_{max.}$ and the intensity of the absorption are determined by several factors, among which are the nature of the substitution in α, β, β' and R, the concentration, the temperature, the nature of the solvent and of the resonating structures of the substance in solution. It is of the greatest importance to check the spectrophotometer before use (13).

* Received February 27, 1953, from the Research Department of Pharmaceutical Laboratories, (Dr. J. C. Janssen), Turnhout, Belgium.

Digitaloid substances dissolved in water show $\lambda_{max.}$ which on the average lie about 3 to 4 mμ higher than in an alcohol solution. Since, on the other hand, the solubility of these substances in water is often too low for analytical purposes, we selected aqueous alcohol (50% by volume) as solvent for the determination of U. V. spectra of digitoxin and related compounds. We found the reproducibility to be better than with 95% alcohol.

The determination of these spectra at 20± 2° for five different undried digitoxin samples in 3 mg. per 100 cc. solution, gave the results shown in Table I.

TABLE I.—DIGITOXIN SPECTRA

Digitoxin[a]	50% EtOH Max. mμ	E (1%, 1 cm.) ($\lambda_{max.}$)	$\frac{E_{210\ m\mu}}{E_{220\ m\mu}}$
S	219.0	226.5	0.91
E	220.0	200.7	0.80
M	220.0	202.8	0.80
U. S. P.	220.0	193.1	0.80
L	219.0	191.1	0.82
$\bar{m}$		202.8	
σ		13.9–6.9%	
ϵ		6.3–3.1%	

[a] For the digitoxin samples "L," "M," and "S" we are indebted to Dr. K. K. Chen (Indianapolis), Dr. Küssner (Darmstadt), and Prof. A. Stoll (Basel). By "U. S. P." is meant the "U. S. P. Digitoxin Reference Standard" and by "E" digitoxin Eupharma.

For three independent determinations, maximal variations were less than 1% in all cases. The recorded differences for the E (1%,1 cm.) values in the five samples were significant in every instance.

These differences can be partially accounted for. First, the samples contain probably different amounts of water, ethanol, or methanol. It is, however, doubtful whether loss on drying can be considered as an exact analytical method for determining this amount. We observed frequently that dried samples show lower absorption than undried ones, which could be due to partial decomposition. It is further known that digitoxin decomposes with partial sublimation at high temperatures. In any case we believe that analysis of previously dried samples often leads to erroneous results.

Second, the presence of impurities alters the U. V. spectra of digitoxin in the following way: (*a*) aglycones increase the absorption and *D. purpurea* glycosides decrease it, without influencing the position of $\lambda_{max.}$; (*b*) gitoxin, allogitoxin and allodigitoxin show spectra differing only slightly from digitoxin; (*c*) isoaglycones and isoglycosides, probable impurities, due to the frequent use of KOH in one of the last purification steps, show no appreciable absorption down to 200 mμ; (*d*) slightly colored digitoxin samples frequently lose their $\lambda_{max.}$ in the U. V. region due to the end absorption of the coloring matter. The E_{210}/E_{220} coefficient is always increased in these cases; (*e*) digotoxose as such shows no appreciable absorption in the U. V. region considered.

Third, the influence of resonating structures of the chromophore must be appreciable. Some observations point in this direction. When solutions of digitalis aglycones or glycosides are treated with acids or alkalies, the U. V. absorption increases or decreases, respectively, without appreciable influence on the position of $\lambda_{max.}$. Refluxing on a water bath a mixture containing 5 mg. digitoxin, 30 cc. of alcohol 95%, 70 cc. of H_2O and 0.08 equiv. of HCl per 100 cc., the spectra of samples of 5 cc., neutralized with 4 *N* NaOH and diluted in such a way that the O. D. max. falls between 0.5 and 0.8 in 50% alcohol solution, give results shown in Table II.

A slight correction must be applied to the observed values of Table II, due to the end absorption of the solvent in the same experimental conditions. This can be done by determining the approximately constant O. $D._{210\ m\mu}$/O. $D._{220\ m\mu}$ ratio for different O. $D._{220\ m\mu}$ values of the solvent after boiling and the O. $D._{210\ m\mu}$/O. $D._{220\ m\mu}$ ratio of the "boiling mixture" before boiling.

It is clear that the nature of the end absorption of the solvent depends largely on the purity of the ingredients. In this experiment, the correction was applied by means of the following ratios:

Solvent after boiling: O. D. 210/O. D. 220 = 3.2 ± 0.5 for O. $D._{220\ m\mu}$ = 0.1 to 0.8.

"Boiling mixture" before boiling: O. D. 210/O. D. 220 = 0.85 ± 0.02.

With this correction applied it was apparent that, without any appreciable change of $\lambda_{max.}$, the E (1%, 1 cm.) values in the two experiments described in Fig. 2 and Table II (B and C) increased with some 123% and 146.5%, respectively, which means an increase of log ϵ from 4.185 to 4.533 and 4.574, respectively. After a boiling time of ninety minutes the increased values became practically constant. Boiling with NaOH under identical conditions resulted in a 100% decrease of the E (1%, 1 cm.) value after one hundred and twenty minutes.

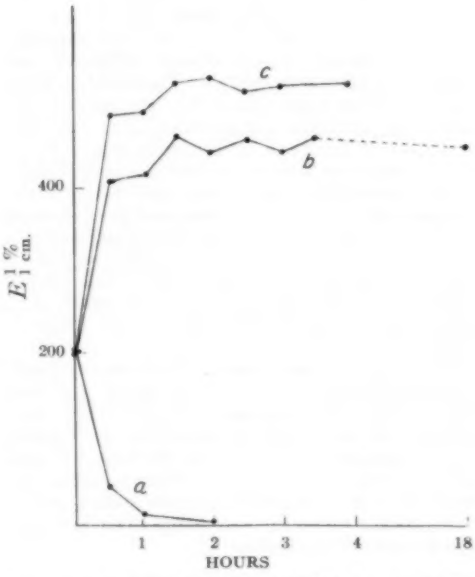


Fig. 2.—Influence of HCl and NaOH on the ultraviolet spectrum of digitoxin.

TABLE II.—INFLUENCE OF HCl ON DIGITOXIN SPECTRA

	c	b	Δbc
E(1%, 1 cm.)	515 (+186%)	451 (+150%)	14.2%
σ	13 (2.5%)	18 (4%)	
ε	6 (1.2%)	7.5 (1.5%)	
n	5	6	
E(1%, 1 cm.) corr.	493 (+146.5%)	446 (+123%)	10.2%

The same phenomenon was observed with a number of other aglycones and glycosides. In colored samples the $\lambda_{max.}$, absent due to end absorption, often reappeared after treatment with HCl.

The disappearance of these U. V. spectra under the influence of alkali has been investigated before by different authors. A summary of the proposed reaction mechanisms resulting in the formation of isoaglycones and isoglycosides can be found in articles by Canbäck (14) and by Langejan (15).

As there is no adequate proof that the migration of the $\Delta^{\alpha,\beta-}$ double bond to the $\Delta^{\beta,\gamma}$-position actually takes place, and considering the evidence (Jacobs, 1929) that the rate of formation of the ether between C_{14} and γ is greater than the hydrolysis of the butenolide structure, we believe that the reaction III → V → VII (Fig. 3) probably plays an important role in the formation of the isocompounds of type VII.

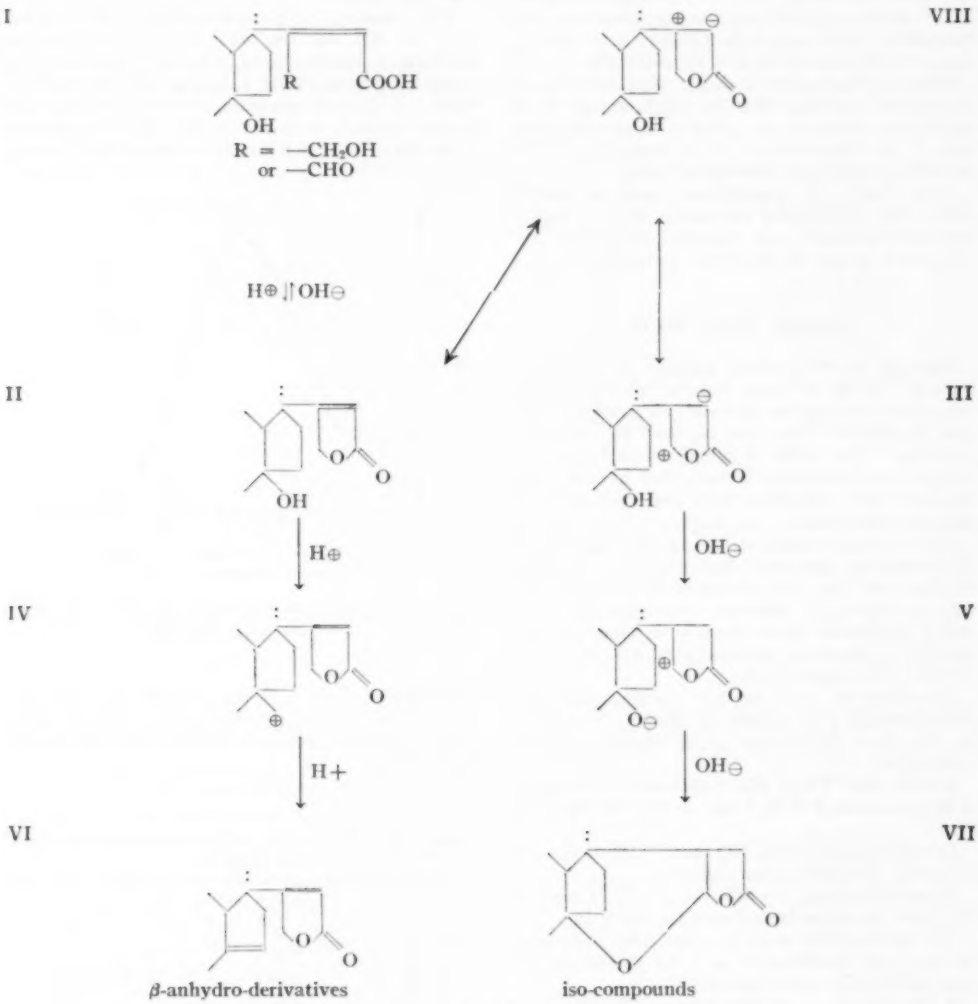


FIG. 3.

Very little is known concerning the influence of acids on the aglycone portion of digitalis glycosides. Under drastic conditions anhydro derivatives (VI) are formed (II → IV → VI: Fig. 3). The possibility remains that open lactones (hydroxy- or aldehydo-acids) exist as such and in varying amounts in dissolved $\Delta^{\alpha,\beta}$-butenolides and are closed again by acid treatment. The results of the lactone titration as described by Windaus, Stoll, and Küssner (16) do not exclude this possibility. Nor is there anything known about the importance of probable resonating structures, such as III or VIII (Fig. 3).

Canbäck (14) attributed the color reaction with *m*-dinitrobenzene to the formation of very reactive and transient carbanion, charged in the α-position of the butenolide group.

There is some evidence that lactone formation from open hydroxy- or aldehydo-acids results in stronger absorption without great change in the position of $\lambda_{max.}$: β, β' substituted acrylic acids all show $\lambda_{max.}$ between 210 and 227 mμ with log ϵ = 4.1 ± 0.1 in EtOH, and on the other hand the U. V. spectrum of β-(*p*-acetoxycyclohexane)-$\Delta^{\alpha,\beta}$ butenolide was reported by Ruzicka, *et al.* (17), as: $\lambda_{max.}$ = 217 mμ and log ϵ = 4.6 in EtOH.

There is finally the probable increase in U. V. absorption resulting from the displacement of the equilibrium between the different resonating structures of $\Delta^{\alpha,\beta}$-butenolides (Fig. 3) from III and VIII toward II under the influence of acids.

These facts and possibilities must be kept in mind while interpreting the results of U. V. spectra determination and color reactions due to the $\Delta^{\alpha,\beta}$ butenolide group of digitaloid compounds.

COLOR REACTIONS

Although a tremendous amount of qualitative information on a large number of colorimetric reactions with digitalis glycosides is available, very few quantitative data can be used for analytical purposes. Very often a wrong wave length was selected and important factors, such as time, temperature, and impurities were neglected or insufficiently controlled.

The general aspect of the spectra for the *m*-dinitrobenzene reaction (Raymond), as described by Canbäck (14), was confirmed by Rowson (18): $\lambda_{max.}$ = 620 mμ for digitoxin. The reported extrapolated absorption values to t = 0 however were determined by these authors as E (1%, 1 cm.) = 140 and 192, respectively.

The reported data on the reaction with 3,5-dinitrobenzoic acid cannot be directly compared, as they were determined under slightly different conditions.

Kedde (19): Filter S53, stable color after thirty to fifty minutes, E (1%, 1 cm.) = 64.0 for digitoxin, $3H_2O$.

Langejan (15): Filter 550 mμ, E (1%, 1 cm.) = 63.1 after ten minutes for digitoxin.

Rowson (18): $\lambda_{max.}$ = 535 mμ, E (1%, 1 cm.) = 85.0 after six to twelve minutes for digitoxin.

The same caution must be taken while comparing the reported quantitative data for the picric acid (Baljet) reaction with digitoxin:

Kaern (12): $\lambda_{max.}$ = 486–492 mμ; E (1%, 1 cm.) = 214 after fifteen to forty-five minutes.

Kennedy (20): $\lambda_{max.}$ = 495 mμ, E (1%, 1 cm.) = 227.5 after thirty minutes.

Neuwald (21): F 490 mμ, E (1%, 1 cm.) = 214 ± 2 after thirty minutes.

Rowson (18): $\lambda_{max.}$ = 490–500 mμ, E (1%, 1 cm.) = 190 after ten to twenty minutes.

The well-known investigations of Bell and Krantz (22) are of limited value due to the unfortunate selection of a wrong wave length (525 mμ). The same wave length is proposed in U. S. P. XIV.

Well-founded criticism concerning the reported data for the Keller-Kiliani reaction can be found in an article by Rowson (18). This investigator reported two $\lambda_{max.}$ for digitoxin: 590 mμ (E (1%, 1 cm. = 118) and 470 mμ.

Using a checked Beckman DU spectrophotometer, we determined the absorption spectra obtained by the action of the Baljet and Keller-Kiliani reagents on a number of digitoxin samples and allied aglycones and glycosides. For the Baljet (alkaline picrate) reaction the method described by Rowson (18) was adopted.

Five undried digitoxin samples all showed $\lambda_{max.}$ = 490 mμ, the color at this wave length reaching a maximum of intensity after four to seven minutes at 20° ± 2, fading very slowly afterward. A typical spectrum, determined after five to ten minutes, is shown in Fig. 4. The greatest value for ϵ, recorded for three independent determinations of E (1%, 1 cm.) at 490 mμ, was less than 1%. The differences between the E (1%, 1 cm.) values as shown in Table III are significant in all cases.

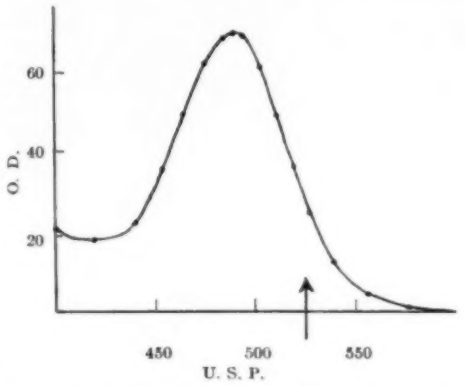


Fig. 4.—Spectrum of the Baljet (modified Rowson) reaction with digitoxin.

TABLE III.—SPECTRUM OF BALJET REACTION WITH DIGITOXIN

	E (1%, 1 cm.) Max.	
S	236.9	
E	206.7	$\bar{m}$ 210.9
M	228.0	σ 25.6 (12.3%)
U. S. P.	216.8	ϵ 11.7 (5.6%)
L	170.9	

The values for the same digitoxin samples given in Table IV, refer to the colorimetric assay according to the method described in U. S. P. XIII, p. 641 (Keller-Kiliani), (20 ± 2°). A typical spectrum (Fig. 5) can be drawn as follows:

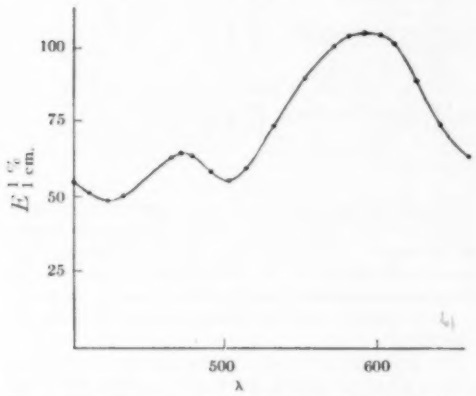


Fig. 5.—Spectrum of the Keller-Kiliani reaction (U. S. P.) with digitoxin after ninety minutes.

spectra, Baljet, and Keller-Kiliani reactions) are interesting:

First, for the five undried digitoxin samples examined the standard deviation around the mean is smaller in the case of the Keller-Kiliani reaction than for the U. V. spectra and the Baljet reaction, although the individual probable error is highest in the Keller-Kiliani test. This seems to indicate that the five samples differ less in respect to their digitoxose content than to the nature and concentration of the butenolide ring. It is possible to demonstrate that the described Keller-Kiliani test is very specific for the sugar-moiety of the digitalis glycosides and is but little influenced by the nature of the butenolide structure. The following results (Table V) confirm the opinion of Stoll (23) that in the experimental conditions of the test, the hydrolyzed digitoxose molecules are responsible for the color reaction. Digilanidobiose, remaining unhydrolyzed, explains the fact that lanatosides and *purpurea*-glycosides show only two-thirds the color intensity of digitoxin, gitoxin, or digoxin. In Table V the maximum molar extinction values ($\epsilon_{max.}$) for several glycosides[1] at 585–590 mμ, $\lambda_{max.}$ in all cases, are shown. No important differences could be detected between the Keller-Kiliani spectra of these com-

TABLE IV.—SPECIFIC ABSORBANCIES, KELLER-KILIANI U. S. P. XIII, UNDRIED DIGITOXIN IN 6 MG. % CONCENTRATION

	E (1%, 1 cm.) 590 mμ	E (1%, 1 cm.) 500 mμ	E (1%, 1 cm.) 470 mμ
S	112.90 ± 0.8	60.8 ± 1.2	72.1 ± 2.5
E	104.94 ± 0.5	54.1 ± 0.7	62.0 ± 0.5
M	105.45 ± 0.8	54.6 ± 0.7	63.6 ± 0.5
U. S. P.	101.65 ± 0.35	52.5 ± 0.35	62.5 ± 0.18
L	105.52 ± 1.3	54.6 ± 1.0	65.0 ± 3.0
m	106.1	55.3	65.0
	4.3 (4%)	2.9 (5.2%)	16.9 (26%)
	2.0 (1.8%)	1.3 (2.4%)	7.7 (11.8%)
	65–100 min.	65–100 min.	90 minutes

(*a*) $\lambda_{max.}$ = 590 mμ with increasing intensity during the first sixty-five to seventy minutes, fading slowly after a constant period of some twenty minutes.

(*b*) $\lambda_{max.}$ = 500 mμ with increasing intensity during the first seventy to eighty minutes, fading slowly after a constant period of some thirty minutes.

(*c*) $\lambda_{max.}$ = 470 mμ, increasing very rapidly during the first one to three minutes and decreasing immediately afterward. When the optical densities at 470 mμ are plotted against the logarithm of the time, a straight line was obtained in all cases, suggesting a simple reaction which showed to be very thermosensitive.

(*d*) $\lambda_{max.}$ = 415 ± 5 mμ with maximum absorption after fifty to one hundred minutes. When concentration was plotted against optical density for different wave lengths, straight lines were obtained for 500 and 590 mμ, flattened or sigmoidal curves for 470 mμ values, determined after a fixed time or extrapolated to $t = 0$, and for 410–420 mμ.

Several aspects of the comparison of the results obtained with the three analytical methods (U. V. pounds and that of digitoxin, although the kinetics of the reactions involved differ slightly. In all cases $\epsilon_{590\ m\mu}/\epsilon_{500\ m\mu} = 1.8 \pm 0.1$. The Keller-Kiliani spectrum for digitoxose itself is also similar, but the molar extinction values at 590 mμ are surprisingly low and increase from 18,000 to about 23,000 with the concentration increasing from 1 to 8 mg. %.

TABLE V.—MAXIMUM MOLAR EXTINCTION VALUES FOR SEVERAL GLYCOSIDES

Digitoxin	3 × 27,060 ± 20
Digoxin	3 × 26,943 ± 40
Purpurea glycoside	
A	2 × 26,900 ± 50
C	2 × 27,800 ± 75
Lanatoside	
A	2 × 27,250 ± 100
B	2 × 30,850 ± 80
C	2 × 25,800 ± 80
m	27,500
σ	500 (1,8%)
ϵ	190 (0,7%)

[1] Obtained through the courtesy of Prof. A. Stoll (Basel).

TABLE VI.—EXTINCTION VALUES FOR DIFFERENT AGLYCONES AND GLYCOSIDES RELATED TO DIGITOXIN

E (1%, 1 cm.)	U. V. Spectra 50% EtOH $\lambda_{max.}$ 219–221 Mμ	Baljet-Rowson 490 Mμ $\lambda_{max.}$	Keller-Kiliani U. S. P. XIII $\lambda_{max.}$ 585–590 Mμ
Digitoxigenin	420 ± 5	504.1	.....
Digitoxin (5 samples)	202.8	210.9	106.1:3 = 35.37
D. purpurea glycoside A	166 ± 1	194 ± 1	58 ± 1:2 = 29.0
Lanatoside A	155 ± 2	190 ± 5	55 ± 2:2 = 27.5
Lanatoside B	160 ± 5	195 ± 1	61.5 ± 1.5:2 = 30.75
Digoxigenin	340 ± 5	420 ± 2	.....
Digoxin	200 ± 2	227 ± 1.5	103.5 ± 1.5:3 = 37.83
D. purpurea glycoside C	160 ± 0.5	185 ± 1	59.0 ± 1.5:2 = 29.5
Lanatoside C	140 ± 5	164 ± 2	51.5 ± 1.5:2 = 25.75

Second, neglecting the comparatively small errors of the mean, calculation of the correlation coefficient r shows that the correlation is significant

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{[\Sigma(x - \bar{x})^2(y - \bar{y})^2]}}$$

($P < 0.1\%$) between the extinction values at $\lambda_{max.}$ for U. V. spectra *vs.* Baljet reaction and *vs.* Keller-Kiliani reaction, and insignificant between the value for Baljet reaction *vs.* Keller-Kiliani reaction. This could be explained by assuming the presence of different amounts of allocompounds in the samples.

The same correlations are however significant in the three cases considered (U. V. *vs.* B, U. V. *vs.* K. K., B *vs.* K. K.) when the extinction values for different aglycones and glycosides related to digitoxin are compared as shown in Table VI and Fig. 6.

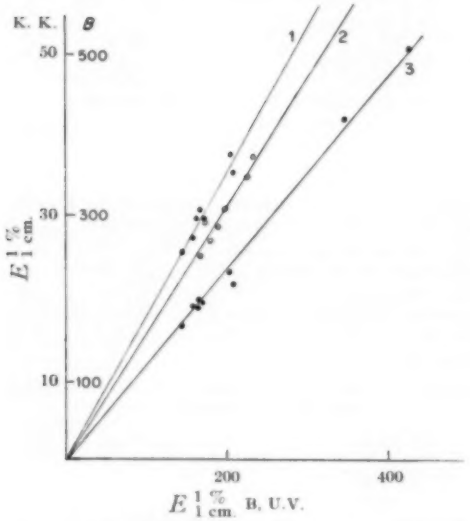


Fig. 6.—Correlations between the extinction values of the Keller-Kiliani reaction (K. K.), the Baljet reaction (B), and the ultraviolet spectra (U. V.) for different *Digitalis* aglycones and glycosides. 1—U. V. *vs.* K. K.; 2—B *vs.* K. K.; 3—U. V. *vs.* B.

From the straight lines in Fig. 6, representing slopes which are not significantly different from the slopes of the statistical lines of best fit, the following constant ratio between the extinction values for the three analytical methods described can be drawn:

U. V./Baljet 490 mμ/Keller-Kiliani 590 mμ = 100/89/67.

No attempt is made to explain the surprising nature of these slopes, as it was realized that the values on which the slopes are based are of limited significance, due to the fact that they were determinted on only one sample in six out of nine cases. The exceptions being digitoxin (five samples), digoxin, and lanatoside C (two samples each).

SUMMARY

1. The possibilities and limitations of some chromatographic and spectrophotometric techniques, used in the analysis of digitoxin and related substances, have been discussed.

2. Evidence is presented for the fact that the physicochemical properties of pure digitoxin are still largely unknown.

REFERENCES

(1) Janssen, P., *Drug Standards* **21,** 177(1953).
(2) Stoll, A., *Helv. Chim. Acta,* **34,** 1470(1951).
(3) Svendsen, A. B., and Jensen, K. B., *Pharm. Acta, Helv.,* **25,** 241(1950).
(4) Jaminet, F., *J. pharm. Belg.,* **7,** 169(1952).
(5) Haberman, E., Müller, W., and Schreglman, A., *Arzneimittel-Forsch.,* **3,** 30 (1953).
(6) Vastagh, G., and Turson, J., *Pharm. Zentralhalle,* **92,** 88(1953).
(7) Hassal, and Martin, *J. Chem. Soc.,* **1951,** 2766.
(8) Mesnard, and Devèse, *Ann. Pharm. Franç.,* **9,** 157 (1951).
(9) Okada, M., Yamada, A., and Kometani, K., *J. Pharm. Soc. Japan,* **72,** 930(1952).
(10) Ulrix, F., *J. pharm. Belg.,* **3,** 15(1948).
(11) McChesney, E., Nachod, F., Auerbach, M., and Laqueur, F., THIS JOURNAL, **37,** 364(1948).
(12) Kaern, M., *Dansk Tidskr. Farm.,* **26,** 92(1952).
(13) Demoen, P., and Janssen, P., *J. pharm. Belg.,* **7,** 80 (1952).
(14) Canbäck, T., *Svensk Farm. Tidskr.,* **54,** 201(1950).
(15) Langejan, M., *Pharm. Weekblad,* **86,** 593(1951).
(16) Küssner, W., *Arch. Pharm.,* **274,** 41(1941).
(17) Ruzicka, *et al., Helv. Chim. Acta,* **29,** 473(1946).
(18) Rowson, J., *J. Pharm. and Pharmacol.,* **4,** 814(1952).
(19) Kedde, D. L., Bijdrage tot het chemisch onderzoek van digitalispreparaten, Ydo, Leyden, 1946, p. 53.
(20) Kennedy, E. E., THIS JOURNAL, **39,** 25(1950).
(21) Neuwald, F., *Arch. Pharm.,* **283,** 93(1950).
(22) Bell, F., and Krantz, J. C., *J. Pharmacol. Exptl. Therap.,* **83,** 214 (1945); **87,** 198(1946); **88,** 14(1946); **89,** 143(1947); **91,** 130(1947). THIS JOURNAL, **35,** 260(1946); **37,** 297(1948); **38,** 107(1949); **39,** 319(1950).
(23) A. Stoll, *Helv. Chim. Acta,* **16,** 1073(1933).

1954 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1954 Chilean Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1954 Award, nominations must be received on or before January 1, 1954.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council. The present committee includes Justin L. Powers, chairman; Paul Block, Louis Gershenfeld, Harvey B. Haag, Lloyd M. Parks, Heber W. Youngken, and Charles O. Wilson.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

The award is now in its sixth year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada. The fourth award was shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex. Dr. Vernon H. Wallingford, Mallinkrodt Chemical Works, St. Louis, Missouri, was chosen as the recipient of the fifth award which was conferred during the 1953 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Book Notices

Blood Cells and Plasma Proteins. JAMES L. TULLIS, Editor. Academic Press, Inc., 125 E. 23rd St., New York, 1953. xxi + 436 pp. 15.5 x 23.5 cm. Illustrated. Price $8.50.

This volume is No. 2 in a series of Memoirs of the University Laboratory of Physical Chemistry Related to Medicine and Public Health of Harvard University, Cambridge, Mass., and deals with the present status of knowledge concerning blood and its component parts. The work is based on seminars delivered at Harvard during February, March, and April of 1951.

The first four chapters are contributed by Dr. Edwin J. Cohn, of Harvard University. The first of these chapters presents an historical review of the development of knowledge about blood. In the second chapter the plasma proteins and their interactions with neutral salts and their solubility in alcohol-water mixtures at low temperatures are discussed. The next two chapters present the recent characterization of proteins based on their interactions with each other, with heavy metals, with specific polysaccharides, and with blood cells.

In the second section the factors concerned with blood coagulation are presented in a series of five chapters by different authors. In the first chapter evidence is presented concerning the chemical nature of prothrombin and certain of the accelerator substances, followed by a chapter giving particular attention to the role of the accelerator substances in hemostasis. In the third chapter fundamental observances on the functions of the various clotting factors in isolated systems of purified reagents are covered. This is followed by a chapter involving basic observances on the interactions between thrombin and fibrinogen. The fifth chapter of this section presents new data on the role of blood platelets in the coagulation mechanism as well as techniques for the long-term preservation of these cells.

In the third section, the components of human blood concerned with immunity are covered in a series of chapters discussing the nature of immune antibodies in human gamma globulin, physical characteristics of the gamma globulins, and the cellular sources of immune antibodies.

The next section includes chapters reviewing, in a general way, the origin, properties, and functions of the human red cell, the preservation of red cells, the influence of certain cations on the storage of human erythrocytes, and the enzymology of the erythrocyte.

In the fifth section, four chapters are devoted to the leucocytes beginning with the development of knowledge about white cells and followed by new concepts concerning the role of white cells in bodily defense. In the third chapter of this section, problems involving the histamine content of white cells are discussed. In the final chapter, data are presented based on the application of new spectrophotometric measurements to cytochrome components of leucocytes and other types of cells.

In the sixth section, the enzymes of plasma are discussed and in the seventh and final section, the lipoproteins of blood and other tissues are covered in a series of five chapters.

The book is well documented throughout with references given at the end of each chapter An author index and a subject index are provided. The printing and binding are of good quality.

Bacteriostatic Activity of 3500 Organic Compounds for Mycobacterium Tuberculosis var. Hominis. By GUY P. YOUMANS, LEONARD DOUB, and ANNE S. YOUMANS. Chemical-Biological Coordination Center, National Research Council, Washington, D. C., 1953. 25 x 17.5 cm. v + 713 pp. Formula index and alphabetical index. Price $5.00. (Available from the Publication Office, National Research Council, 2101 Constitution Ave., Washington 25, D. C.)

This volume describes the techniques used in the *in vitro* and *in vivo* screening of 3,500 compounds for their action against the tubercle bacillus together with the results of these tests. The test data reported are essentially negative from the standpoint of finding a suitable chemotherapeutic agent for the treatment of tuberculosis. The data are valuable, however, since they permit certain generalizations to be drawn concerning the effect of chemical structure on biological activity. The publication of these data will serve to inform other investigators working on this problem of what items have been tested and thus prevent duplication of effort by unnecessary testing of the same compounds. It may also serve to stimulate interest in specific compounds which show promise and may suggest the synthesis of new compounds to fill gaps which are brought out in the data presented. The book is divided into five chapters, the first of which is devoted to a discussion of the rationale for the *in vitro* and *in vivo* screening program. In the second chapter the experimental methods employed in the indexing of compounds for their tuberculostatic activity are described. Chapter three is devoted to an interesting discussion of selected data in relation to chemical structure and the last two chapters are devoted to an *in vivo* table and an *in vitro* table, respectively.

Natural Child Spacing. The Body Temperature Method of Child Planning. By M. EDWARD DAVIS. Hanover House, Garden City, N. Y., 1953. 67 pp. 14 x 21 cm. Price $2.

This practical and easy-to-read book by Dr. Davis is well illustrated with many descriptive, two-color charts and diagrams. A section of illustrated case histories which describes and explains this simple method, is an important feature of the book. The last part of *Natural Child Spacing* consists of a number of removable, blank charts for the reader's use when following this advanced method of child planning.

Atomic Energy in Industry. Minutes of Special Conference. National Industrial Conference Board, Inc., 247 Park Avenue, New York 17, New York, October, 1952. 40 sections. 22 x 28 cm. Paper bound. Illustrated. Mimeographed Price $10.

This ring-bound volume is a compilation of the papers presented at a special conference of the National Industrial Conference Board, October 16–17, 1952, in New York City. The participants are outstanding individuals in the different fields of nuclear energy.

Of particular importance to pharmacists are Sections 24 through 29 of the total of 40 sections. These sections deal with (24) Medical and Pharmaceutical Applications of Atomic By-Products: Introduction, Charles Dunham; (25) Medicine and Pharmaceutical Applications, D. A. McGinty; (26) Diagnostic Uses of Radioactive Isotopes, Edith H. Quimby; (27) Use of Radioisotopes in the Field of Medicinal Chemicals Manufacture, Charles Rosenblum; (28) The Therapeutic Use of Radioactive Isotopes, Bernard Roswit; and (29) Medical and Pharmaceutical Applications, D. L. Tabern.

Plants of the Bible. By H. N. and A. L. MOLDENKE. The Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York, 1952. Illustrated. xx + 328 pp. 18 x 27 cm. Price $7.50.

This book is an encyclopedic treatise, citing "chapter and verse" references for 230 drugs. The drugs are listed numerically and in the Table of Contents, and, although the basic arrangement of the Table and the text is alphabetical, consecutive numbers are given to primary and secondary names of plants.

An interesting historical sketch, a description of the Biblical lands, and an explanation of the references in the text form an introductory section.

A chapter on "Unidentified Plant References" indicates the thorough search for material. It includes such statements as "Proverbs 3:18—She is a *tree* of life to them that lay hold upon her. . ." A bibliography, an index to the quoted Bible verses, an extensive general index, and a number of plates are appended.

This book should be a useful reference to students of the title subject.

1953 Medical Progress, A Review of Medical Advances During 1952. MORRIS FISHBEIN, Editor. The Blakiston Co., N. Y., 1953. 301 pp. 16 x 23.5 cm. Price $5.

This first volume in a proposed series is a compilation of reviews by authorities in different medical specialties which include: Cardiology—Heart Disease; Surgery; Infectious Diseases; Arterial Hypertension; Rheumatic Diseases; Nutrition; New and Important Drugs and Medication; Dermatology; Urology; Psychiatry and Psychosomatic Medicine; Obstetrics; Diseases of the Chest; Ear, Nose, and Throat; Gastro-intestinal Disorders; Allergy; and Orthopedic Surgery. The editor adds a short review on medical advances in which he touches briefly on subjects that are treated more fully by other authors in the text and on some subjects that are not mentioned by others.

The book is an excellent review by competent authors who present their reports in different styles, all of which are interesting and with full bibliographies at the end of each section. A general index is appended.

The book should be valuable to those physicians who are unable to cover the medical literature and it should be available to anyone who has need to refer to recent medical progress reports.

Substances Naturelles de Synthèse. Volume VI. LEON VELLUZ, Editor. Masson & Cie, Paris, 1953. 156 pp. Illustrated. 16 x 22.5 cm.

The sixth volume of this French series was prepared by A. Allais, J. Mathieu, A. Petit, P. Poirier, and L. Velluz. It follows the same style and arrangement as the first five volumes, but this volume has a stiff cloth-covered backing and better paper.

Methods of synthesis are given for desoxycholoic acid, glycocholic acid, lithocholic acid, adrenochrome DL-cynurenine, histamine, 17-hydroxy-11-desoxycorticosterone, and DL-2-hydroxytryptophan. General methods discussed are imidazolic cyclization and separations by ion exchange. Examples of the application of the latter are given. The index refers only to the first section of the book which deals with the completely described methods of synthesis.

Methoden der Organischen Chemie. (Houben-Weyl), Vierte Auflage, Band VIII, Sauerstoffverbindungen III, Herausgegeben von EUGEN MULLER. Georg Thieme Verlag, Stuttgart, Germany, 1952. (Agents for the U. S.: Grune and Stratton, 381 Fourth Avenue, New York, N. Y.) xviii + 775 pp. 18 x 26.5 cm. Price *DM* 98.

A completely new and revised fourth edition of this well-known and highly esteemed book is being compiled. The new edition of *Methoden der organischen Chemie* starts with the eighth volume which was published late in 1952. According to present plans, about fourteen volumes, each containing approximately 700 pages, will be required to complete the new fourth edition. This is in marked contrast to the last four-volume third edition, published more than 20 years ago. It is expected that several years may be required to complete the new edition. On the basis of current costs of production, it is estimated that each volume will cost approximately *DM* 100. The final price, however, will be determined at the time of publication of each volume. A ten per cent discount is allowed on advance orders.

The first volume, to be published later, will contain general instructions for laboratory experiments and the conventional methods of organic chemistry such as separation and purification. Analytical methods of organic chemistry will be considered in Volume II. Following the first two volumes, subsequent ones will deal with the physical research methods of organic chemistry. The main body of the work, however, will be concerned primarily with preparative methods. Each volume will be indexed separately, but when the project is completed, an author and subject index to all of the volumes will be provided.

Since the main function of the new edition will be to present significant preparative methods for many organic compounds, the new edition starts with the eighth volume. This volume includes six compre-

hensive chapters, the first of which deals with the peroxides and the second with derivatives of carbonic acid. In the third chapter, the methods for the synthesis and conversion of nitriles, isonitriles, and fulminic acid are described. In the fourth chapter, the carboxylic acids and decarboxylation procedures are covered. The fifth chapter is devoted to esters of carboxylic acids, and the sixth chapter to functional nitrogen-derivatives of the carboxyl group.

A complete author index and subject index are provided and the book is thoroughly documented throughout. Those who have used *Houben-Weyl* as a reference book will be greatly impressed by the scope and content of the eighth volume of the new edition, which is intended to be representative of subsequent volumes to appear during the next few years. All chapters in the eighth volume have been written by well-known specialists of the Bayer Dye Works of Leverkusen and Elberfeld, with the exception of the chapter on peroxides and parts of that on carbonic acid derivatives.

Basic Drugs. U. S. Public Health Service Hospitals and Clinics, 1953. Federal Security Agency, Public Health Service, Bureau of Medical Services, Division of Hospitals. Public Health Service Publication No. 246. iv + 165 pp. 15 x 23 cm. Price 50¢. (For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.)

The basic drugs presented in this book are thought of by those who compiled it as the group from which each station shall choose the items to be regularly stocked in its pharmacy. The development of the handbook has been a cooperative Public Health Service effort which was designed to provide the patient with the best possible drug therapy, and the physician and dentist with carefully selected agents of proved effectiveness. It is also intended to give a standard of comparison for the evaluation of new therapeutic agents, as a reference for the essential pharmacology of basic drugs, and to simplify all drug therapy record keeping.

In the selection of basic drugs, an attempt was made to sort out the smallest number of the best, simplest, and safest medicines currently needed in the ordinary prevention, diagnosis, and treatment of illnesses. The criterion utilized in the selection of these drugs involved as a primary consideration the therapeutic efficacy of the drugs. Within these limits, preference has been given to items listed in The *United States Pharmacopeia*, The *National Formulary, New and Nonofficial Remedies*, and *Accepted Dental Remedies*. Every attempt has been made to avoid unnecessary duplications, and this, in some cases, has perhaps resulted in being somewhat too restrictive. Provison is made, however, for deviations from the basic drugs list, but such deviations are possible only with the written approval of the station pharmacy committee. This requirement does not apply to the procurement of nonbasic drugs for an individual patient in an emergency. No drug, the composition of which is secret, is included in the basic drugs list, and mixtures only rarely and then only when they provide a substantial advantage over the individual components. The book is exceptionally interesting because of the contrast between the basic drugs considered indispensable for the practice of dentistry and medicine in service hospitals and clinics, and the actual number of drugs prescribed and dispensed in the noninstitutional practice of medicine, dentistry and pharmacy.

Historical Metrology. (A New Analysis of the Archeological and the Historical Evidence Relating to Weights and Measures.) By A. E. BERRIMAN. E. P. Dutton & Co., New York, 1953. xvi + 224 pp. Illustrated. 13.5 x 20 cm. $3.75.

Mr. A. E. Berriman has analyzed the archeological and historical data concerning weights and measures and has written a useful and compact little book on this subject. Chapters on Hindu, Chinese, Mesopotamian, Egyptian, Greco-Roman and Palestinian metrology are presented. Of interest to the numismatist and economic historian is the chapter dealing with "Metrological Aspects of Money." Discussed also in this work are premetric metrology in France and aspects of Russian metrology. A succinct chronological history of the metric system is given.

Perhaps the best written and undoubtedly one of the most interesting chapters in the book is the one dealing with historical aspects of English metrology. The last chapter which consists of only two pages is, as the author states, confined to a summary of the "principal differences between U. S. and U. K. metrologies."

The book is well illustrated; it has informative appendices and a valuable bibliography.

Methods and Reactions of Organic Analysis. Volume II. Methods of Identification. LÉON VELLUZ, Editor. Masson & Cie, Paris, 1953. 278 pp. Illustrated. Paper bound, uncut. 17 x 25.5 cm.

This volume, the second of three volumes in French, was prepared by Maurice Pesez and Pierre Poirier with J. Mathieu, A. Petit, and J. Wohlgemuth, and was edited by L. Velluz. The first volume was reviewed in THIS JOURNAL, **41,** 457 (1952).

The text is divided under the following section headings: (1) Alkylation and Arylation, (2) Acylation, (3) Ureas, Urethans, and Allophanates, (4) Amidation, (5) Oximes and Hydrazones, (6) Salts and Addition Compounds, and (7) Diverse Reactions. In addition to general discussions and specific applications of the procedures, a concurrent and extensive bibliography, microphotographs, and tabulated data are included. A general index is appended.

Individual and Community Health. By WILLIAM W. STILES. The Blakiston Co., New York, 1953. 492 pp. 16 x 23.5 cm. Price $6.

The ultimate objective of health education, according to Dr. Styles, who is Associate Professor of Public Health at the University of California, Berkeley, is that of promoting health and preventing disease. Other objectives of health education for college students are those of developing a social conscience and integrating individual and community health, while still other aims of health education are to direct the students' overt behavior and conduct, to prepare the student to accept his

responsibility as an individual and as a group member for action leading to the solution of individual and community health problems, and to recruit and orient students into careers in the various fields of health science.

It is believed that this book may adequately accomplish all that is hoped for by the author. It begins with a chapter on individual and community health, followed by chapters on accidents and violence; wound infections; contact and airborne diseases; general measures of disease control; disorders of nutrition, digestion and metabolism; food sanitation; fecal-borne diseases; water, sewage, and flies; arthropod-borne diseases; animal-borne diseases; environmental and occupational diseases; socio-sexual problems; maternal and child care; disorders of the nervous system; problems relating to medical care; and the challenge to control disease. The titles of the several chapters indicate the extent of the survey of individual and community health problems.

The book is written in a clear and concise style that should make it especially acceptable as a textbook for an undergraduate course for non-professional students. The book is written authoritatively, but at the end of many of the sections are included supplementary suggested readings which serve to enhance its value. The printing and binding of *Individual and Community Health* are of good quality and the book is provided with a comprehensive index.

An Inventory of Social and Economic Research in Health. 1953 ed. Compiled by FREDERICK R. STRUNK. Health Information Foundation, 420 Lexington Ave., New York 17, N. Y. 180 pp. 15.3 x 23 cm. (paper bound).

This second edition, like the first edition which appeared in 1952, is, in effect, an annotated bibiliography and directory of current and recently completed research projects dealing with the social and economic aspects of health programs and health problems. A compilation of this nature is of great value to all who are interested in this subject. The book contains brief descriptions of over 250 research projects, all of which contribute materially to a total comprehension of disease and of organized means of bringing health facilities and people together. In making the book available, the Health Information Foundation which is sponsored by the drug, pharmaceutical, chemical, and allied industries, has rendered public service of a high order.

Drug Addiction Among Adolescents. Conferences held at the New York Academy of Medicine, sponsored by the Committee on Public Health Relations of the New York Academy of Medicine. The Blakiston Co., New York, 1953. 320 pp. 16 x 23.5 cm. Price $4.

Two conferences on drug addiction among adolescents have been held during the past two years, both at the New York Acadeny of Medicine in New York. The first meeting was on November 30, 1951, and the second on March 13, 1952. This book is composed of records of the discussions during these two conferences. The conference proceedings indicate that there is no easy solution for the problem of drug addiction among adolescents. The fact is brought out, however, that the problem is not one of moral degeneracy or a failure of law enforcement, but rather one symptom of serious deprivations of many children living in large and crowded cities. These conditions can be corrected only by supplying the basic needs of children which large urban centers fail to provide, such as adequate housing, modern and adequately manned schools, appropriate recreational facilities, freedom from racial and religious discrimination, opportunities to achieve social and emotional maturity by sharing in community activities under responsible leadership, and exposure to experiences which enhance spiritual values. This book undoubtedly is a valuable contribution to a better understanding of the causes and effects of drug addiction among young people.

Encyclopedia of Chemical Technology. Edited by RAYMOND E. KIRK and DONALD F. OTHMER, Vol. X, Pentacene to Polymethine Dyes. The Interscience Encyclopedia, Inc., New York, 1953. xvi + 976 pp. Illustrated. 20 x 27 cm. Price $25.

The first volume of this important series was published in 1947 and was described and reviewed soon after its appearance, in THIS JOURNAL, **37,** 130(1948). Volume 10 consists of treatises on subjects from pentacene to polymethine dyes. Of special interest to readers of THIS JOURNAL would probably be a section on pharmaceuticals, contributed by H. M. Merker and A. R. Whale, of Parke, Davis and Company. This section discusses chemical and related aspects of the pharmaceutical industry in general, and presents a good picture of the development of the industry up to the present time.

Another section, devoted to perfumes, synthetics and isolates, is also of special pharmaceutical interest. It was written by P. Z. Bedoukian, of Fabergé, Inc., and presents a good survey of the field and includes much general information concerning the nature of perfumes and of the chemicals used in their fabrication. Other typical articles include titles such as petroleum; phenol and phenols; the phase rule; phenolphthalein; phthalic acids; pigments; phosphoric acids and phosphates; photoelectric cells; photography; plant growth substances; and polarimetry. This volume constitutes a valuable addition to those previously published. The design of the book is the same as the one originally established for volume I.

Chemical Control of Insects. By T. F. WEST, J. ELIOT HARDY, and J. H. FORD. John Wiley & Sons, Inc., New York, 1953. xi + 211 pp. 14 x 19 cm. Price $3.25.

This small book presents a good survey of the main insecticides, including descriptions of the materials, how they are used, and the particular insects against which they are effective. The text is divided into the following chapter headings: The Insect, Outline of Pest Control, Fumigation, Rotenone and Related Compounds, Arsenical Compounds, Petroleum Oils, Coal Tar Derivatives, Miscellaneous Insecticides, Soil Insecticides, Pyrethrum, Lethane and Thanite, Chlorinated Persistent Insecticides, Repellants and Attractants, and Weed Control. The text is well documented with references at the end of each chapter and with author and subject indexes appended. Structural formulas and pictures are included.

The capacity of pyrethrum flowers to kill insects was recognized at the beginning of the 19th century, and the authors observe that "No synthetic chemical available and suitable for the purpose exhibits, at anything like equivalent concentrations, the 'knockdown' properties to insects of that complex mixture of compounds known as the pyrethrins." The book is a handy reference for the gardner or chemist.

Organic Chemistry—An Advanced Treatise. Vols. III and IV. Edited by HENRY GILMAN. John Wiley & Sons, Inc., New York, 1953. Vol. III: 16 x 23.5 cm. x + 580 pp. + xxxvii p. index. Price $8.75. Vol. IV: 16 x 23.5 cm. x + pp. 581–1245 + xxxviii p. index. Price $8.75.

Volumes III and IV of Gilman's *Organic Chemistry* are designed to supplement and more nearly complete the first two volumes of this important treatise, the second editions of which were published in 1943. Gilman and the collaborators who are specialists in the several branches of the science of organic chemistry, have completed a general treatise suitable for instruction at the graduate level and for use as a reference to the many subjects covered. The contributors to Volumes III and IV, as to the previous two, are internationally known as authorities in the special field of which they write. The scope is so broad that it can best be visualized by listing the contents of the two volumes.

In Volume III one finds the following chapters: The Study of Organic Reaction Mechanisms; Applications of Infrared and Ultraviolet Spectra to Organic Chemistry; Lipids; Organic Dyes; Some Aspects of Chemotherapy; Antibiotics.

The contents of Volume IV include: The Terpenes; Heterocyclic Chemistry; Starch; Chemistry of Explosives; Reactions of Organic Gases Under Pressure; Oxidation Processes.

In nearly all phases of pharmaceutical research organic chemistry plays one of the most important roles. Parts of Gilman's *Organic Chemistry* will serve adequately for general and specialized instruction at the graduate level in certain phases of pharmaceutical chemistry. The chapters on chemotherapy, antibiotics, and heterocyclic compounds, should be of special interest to those in the pharmaceutical field.

Volumes III and IV follow essentially the same design as the first two volumes. Each chapter includes a table of contents, and each volume includes an index to both volumes. The book is adequately documented throughout and in addition general references are provided at the end of each chapter. The printing and binding are of good quality.

Non-Aqueous Solvents. By LUDWIG F. AUDRIETH and JACOB KLEINBERG. John Wiley & Sons, Inc., New York, 1953. xii + 284 pp. 15.5 x 23.5 cm. Illustrated. Price $6.75.

Much experimental work has been done on the physical and chemical properties of nonaqueous solutions, but relatively little has been reported on how nonaqueous solvents can be used to effect chemical reactions and syntheses. The authors of this book have aimed to stress the latter utilization of these solvents. They have been "guided by considerations which would appeal more specifically to those among our professional colleagues who are particularly interested in synthetic chemistry." This is indicated by the observation that the potential uses of acetic acid are especially interesting in analytical chemistry. Although the preface to the book ends with the date November, 1952, the last reference in the bibliography at the end of the chapter on Acetic Acid, including Reactions in Anhydrous Acetic Acid, is to a thesis written in 1949.

The text is a concise, readable, presentation of fundamental principles, information, and applications, which can be used by beginners and advanced students. The subject matter is divided into subgroups under the following chapter headings: Properties of Solvents; Acids and Bases; Liquid Ammonia as a Solvent and Dispersion Medium; The Nitrogen System of Compounds; Reactions in Liquid Ammonia—Experimental Techniques and Some Important Type Reactions; Metal-Ammonia Solutions; Nitrogen-Containing Solvents; Acetic Acid; Sulfuric Acid; Hydrogen Fluoride; Liquid Sulfur Dioxide; Acid Chlorides; Halogens and Interhalogens as Solvents; and High Temperature Systems.

The applications described in the text are clearly presented and appear to benefit from the practical working knowledge of the authors. An index is included. As is the case in many specialized areas, certain parts of the text, especially the analytical applications, are ready for a revision in the light of the recent reports of work in the field of titrations in anhydrous acetic acid.

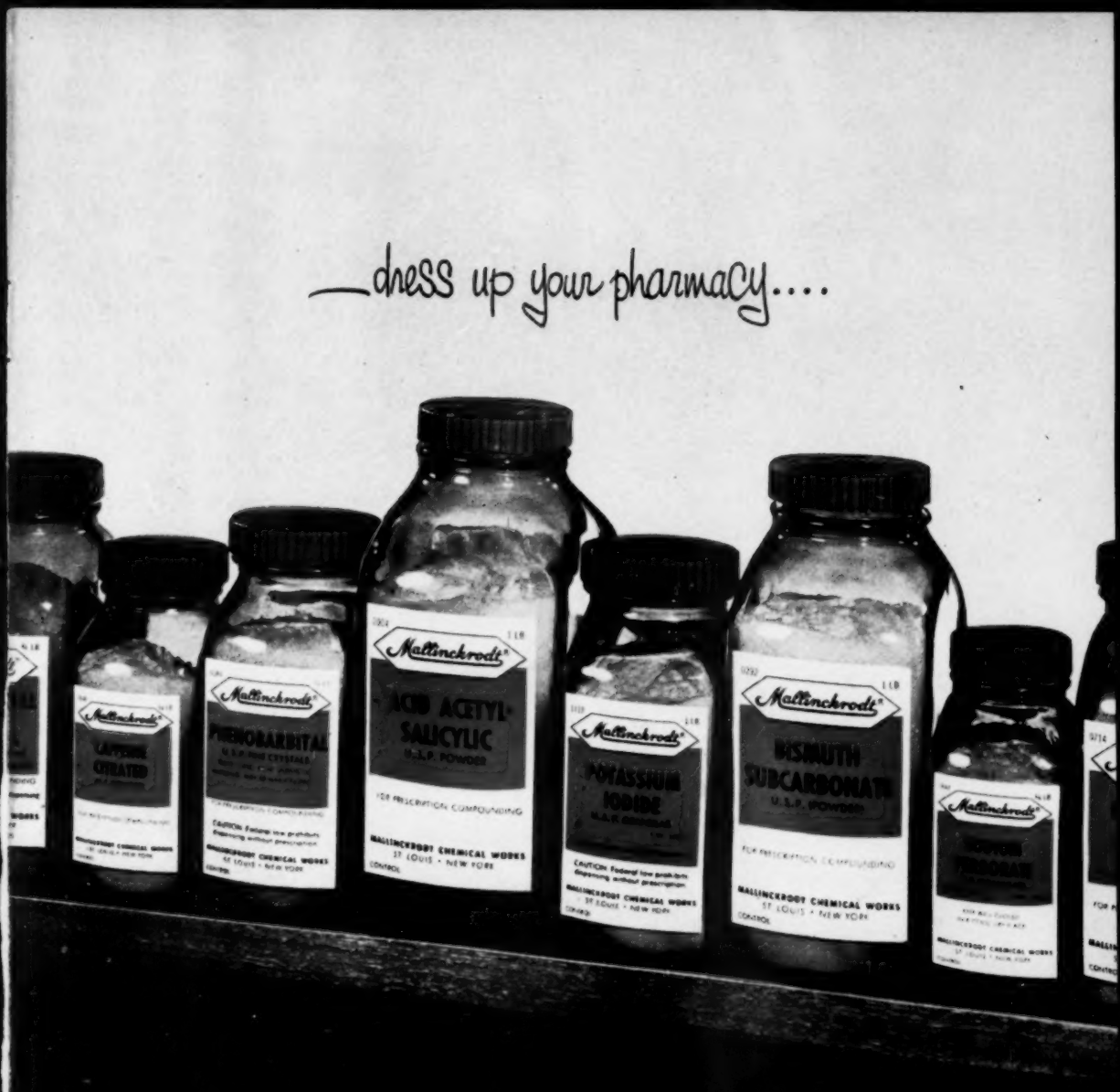
The Suprarenal Cortex. J. M. YOFFEY, Editor. Proceedings of the Fifth Symposium of the Colston Research Society held in the University of Bristol, April 1–4, 1952. (Vol. V, Colston Papers). Academic Press, Inc., Publishers, New York, 1953. 232 pp. 19.5 x 26 cm. Price $6.80.

The papers contained in this volume form a summary of the present state of research in many of the recent investigations of the powerful and widespread effects of the steroid hormones, extending into almost every part of the body and influencing many of its important mechanisms. The author has brought together in this one volume much information which has heretofore been scattered among numerous journals in many branches of science.

Several chapters in the book are devoted to the chemistry of ACTH, the physiology of the suprarenal cortex and its effects on physical and mental processes. Discussions on the recent developments in the clinical uses of cortical steroids, particularly in relation to rheumatic diseases, are presented in the last chapter of this volume.

1952 Year Book of Drug Therapy. HARRY BECKMAN, Editor. The Year Book Publishers, Inc., Chicago, 1952. 606 pp. 13.5 x 20 cm. Illustrated. Price $5.50.

This book is composed of an extensive series of abstracts from the medical literature published during the period August, 1951 to August, 1952. All the abstracts include full references to the original publications, and complete author and subject indexes are appended. The book should be useful to anyone interested in short reviews of the significant developments in general medicine and in drug therapy. The 1951 Year Book was reviewed in THIS JOURNAL, **41**, 338(1952).



...with Mallinckrodt PRESCRIPTION CHEMICALS

in the new Space-saving† STORMOR* bottles—

ask your wholesaler for "STORMORs"...

(Available in many 1 lb. and ¼ lb. sizes, except liquids)

MALLINCKRODT CHEMICAL WORKS • ST. LOUIS • NEW YORK

CHICAGO • CINCINNATI • CLEVELAND • LOS ANGELES • PHILADELPHIA • SAN FRANCISCO • MONTREAL • TORONTO

*Trade Mark

†CONSIDER THESE OTHER ADVANTAGES: ✓EASIER TO HANDLE ✓LABEL ALWAYS FACES FRONT ✓BETTER VISIBILITY

Taking the Wonder out of Wonder Drugs

Finding a new organic weapon for use in the ceaseless war against disease calls for the patience of Job on the part of the chemist.

The great number of compounds developed during the synthesis of a new wonder drug must be carefully checked for identity and potential value. This is done by isolating the intermediate transition compounds and testing them . . . and two of the major checking requirements are refractive index and melting point.

Was this a simple procedure? Definitely not. Obtaining the refractive index on solids often required melting of the compound, preparing derivatives and even more complicated procedures.

This very problem confronted Maltbie Laboratories, makers of fine pharmaceuticals. What did they do about it? They called in Fisher, of course.

The complete answer was provided with the Fisher Refractometer and Heater Head Attachment. This is the only instrument with which the researcher can run melting point and refractive index at the same time on a wide variety of solid compounds . . . and it is done quickly and conveniently with only a tiny amount of sample.

The researcher in the above photo is running both melting point and refractive index on the glucoside derivative he has prepared prior to the final steps in his synthesis of a new spinal anesthetic . . . and the wonder is gone from another wonder drug.

This is typical of the results that may be expected when Fisher Scientific is given the opportunity to help with laboratory problems. Whether it's instrumentation, method, laboratory planning or equipment supply, whenever you have a problem—take it to Fisher first!

• • •

Plants and Complete Stocks: Pittsburgh, New York, Washington, St. Louis, Montreal, Toronto. Branch Offices: Boston, Buffalo, Chicago, Cleveland, Detroit, Philadelphia.

FISHER SCIENTIFIC

America's Leading Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals